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The WORKING GROUP is comprised of representatives of the U. S. Departments of Agriculture; Commerce; Defense; the Interior; Health, Education, and Welfare; State; Transportation; and Labor; and the Environmental Protection Agency.

The pesticide MONITORING PANEL consists of representatives of the Agricultural Research Service, Animal and Plant Health Inspection Service, Extension Service, Forest Service, Department of Defense, Fish and Wildlife Service, Geological Survey, Food and Drug Administration, Environmental Protection Agency, National Marine Fisheries Service, National Science Foundation, and Tennessee Valley Authority.

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Pesticide monitoring activities of the Federal Government, particularly in those agencies represented on the pesticide MONITORING PANEL which participate in operation of the national pesticides monitoring network, are expected to be the principal sources of data and interpretive articles. However, pertinent data *in summarized form*, together with interpretive discussions, are invited from both Federal and non-Federal sources, including those associated with State and community monitoring programs, universities, hospitals, and nongovernmental research institutions, both domestic and foreign. Results of studies in which monitoring data play a major or minor role or serve as support for research investigation also are welcome; however, the *Journal* is not intended as a primary medium for the publication of basic research. Manuscripts received for publication are reviewed by an Editorial Advisory Board established by the MONITORING PANEL. Authors are given the benefit of review comments prior to publication.

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PESTICIDES IN PEOPLE

Organochlorine Pesticide Residues and Polychlorinated Biphenyls in Human Milk, Colorado—1971-72¹

E. P. Savage, J. D. Tessari, J. W. Malberg, H. W. Wheeler, and J. R. Bagby

ABSTRACT

Organochlorine pesticide residues and PCB's were determined in 40 human milk samples collected from women in rural Colorado during 1971-72. The highest levels found were of *p,p'*-DDE which ranged from 19 to 386 ppb; *p,p'*-DDT ranged from 7 to 109 ppb. β -BHC, *o,p'*-DDT, dieldrin, heptachlor epoxide, and *p,p'*-DDD ranged from a trace to 38, 13, 11, 5, and 5 ppb, respectively, PCB's ranged from 40 to 100 ppb. *p,p'*-DDE and *p,p'*-DDT were found in all milk samples, β -BHC in 87.5%, and PCB's in 20%.

Introduction

Laug *et al.* (5) reported DDT in 30 of 32 human milk samples collected in the Washington, D.C., area in 1951. Since that time, a number of investigators in the United States and other areas of the world have reported organochlorine compounds in individual and pooled human milk samples. Recent interest in the polychlorinated biphenyls (PCB's) has also resulted in several investigators reporting on PCB's in human adipose tissues, plasma, and human milk. Dymont *et al.* (2) were unable to find PCB's in human milk collected from participants living in Texas and New Guinea. Finklea *et al.* (3), in a study of 723 plasma samples collected from volunteers living in Charleston County, S. C., found *p,p'*-DDT and DDE almost universal, DDD in 84%, dieldrin in 63%, and PCB's in 43% of the study participants. Newton and Greene (7) analyzed 67 rural and urban human milk samples collected in Australia for organochlorine pesticides. All samples contained DDT and DDE, 12 contained DDD, and 29 contained dieldrin; they did not report on PCB's.

The current study reports levels of the organochlorine pesticides β -BHC, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDD, dieldrin, heptachlor epoxide, and the industrial pollutant PCB's in 40 human milk samples collected from women in rural Colorado during 1971-72.

Sampling and Analyses

Participants were asked to manually express milk samples into glass tubes equipped with plastic screw caps and teflon liners. The filled tubes were kept frozen until time of extraction.

Analytical standards for the chlorinated pesticides were obtained from the pesticide repository of the U.S. Environmental Protection Agency Laboratory, Perrine, Fla. The Aroclor analytical standards were obtained from the Monsanto Chemical Co., St. Louis, Mo. Solvents were redistilled in glass prior to use. The evaluation, storage, activation, and use of the Florisil followed the recommended procedures described in the "Manual of Analytical Methods" (8).

The extraction procedure used was a modification of those described by Guiffreda *et al.* (4) and Curley and Kimbrough (1). The procedure consisted of 3 parts: (1) isolating the fat from the milk, (2) extracting the Aroclors and pesticides from the fat, and (3) cleaning up the extract.

Whole milk (4.5 g to 24.3 g) was weighed into clean glass centrifuge bottles. Glass wool was added to adhere to the coarse precipitate of the milk solids. After addition of 100 ml of acetone, the sample was shaken manually for 1 minute and centrifuged for 2 minutes. The acetone layer was then transferred to a 1-liter separatory

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funnel. The extraction procedure was repeated three more times with equal volumes of 25 ml of acetone each time. All four acetone extractions were combined in the 1-liter separatory funnel.

Twenty-five milliliters of n-hexane was then added to the coarse precipitate of milk solids, shaken, centrifuged, decanted, and combined with the acetone extracts in the 1-liter separatory funnel. This procedure was repeated, and then a volume of 125 ml of 2% sodium sulfate solution and 50 ml n-hexane was added to the acetone and hexane extracts. The separatory funnel was shaken manually for 1 minute and the lower aqueous layer was discarded. The 2% sodium sulfate washing was again repeated, the lower aqueous layer discarded, and the n-hexane layer poured into a 500-ml concentrator flask. The sample extract was taken through the Florisil procedure as described in the "Manual of Analytical Methods" (8).

Primary identification and quantification of the pesticides was accomplished on a MicroTek 220 Gas Chromatograph equipped with a Ni^{63} electron capture detector and Coulson conductivity detector. Two columns having different resolution characteristics were utilized on all samples. Instrument parameters were as follows:

Columns:	(A) Borosilicate glass, 6' x 1/4", packed with 1.5% OV-17 and 1.95% QF-1 on Gas Chrom Q 100-120 mesh Applied Science
	(B) Borosilicate glass, 6' x 1/4", packed with 4% SE-30 and 6% OV-210 on Gas Chrom Q 100-120 mesh Applied Science
	(C) Borosilicate glass, 6' x 1/4", packed with 3% OV-1—column obtained from Tracor
Detectors:	(A) Electron capture, having 14.5 mc Ni^{63} ionizing source
	(B) Coulson conductivity operated in the chloride mode
Temperatures:	(A) Electron capture Injector 245° C Column 200° C Detector 300° C
	(B) Coulson conductivity Injector 245° C Column 200° C Furnace 820° C Block 230° C
Carrier gas:	High purified nitrogen
Flow rates:	60 cc/min for Column A 100 cc/min for Column B Approx. 60 cc/min for Column C

Primary quantification of the Aroclors was accomplished by thin layer chromatography (TLC). TLC provides a sound approach for the semi-quantitation of the stated PCB's because the various compounds of the series have similar Rf values and therefore produce a single spot. Six percent ethyl ether/n-hexane eluate, in concentrate form, is treated with KOH to effect dehydrochlorination of DDT and DDD to their olefins, thus eliminating the problem of separating these pesticides from the PCB's.

Oxidative treatment is then applied to convert any interfering DDE to *p,p'*-dichlorobenzophenone which has an Rf value different from the PCB's. The PCB's are then determined by thin layer chromatography. The procedure used is a modification of the one described in the "Manual of Analytical Methods" (8) which is based on the method used by Mullhern *et al.* (6).

Gas chromatographic sensitivity limits for *p,p'*-DDT and Aroclor 1254® were 20 and 500 picograms, respectively. TLC sensitivity limits for the Aroclors were based upon visual comparison of sample spot intensity to the intensities of Aroclor standards at 25, 50, 100, and 400 ng/μl. Recovery rates were established by spiking separate milk samples with known amounts of standards as follows: Aroclor 1254® at 0.5 ppm and Aroclor 1260® at 1.25 ppm; β-BHC, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, and dieldrin at 1 ppb; *p,p'*-DDT, α-BHC, aldrin, lindane, heptachlor, and heptachlor epoxide at 4 ppb. These samples were then analyzed utilizing the described procedures. Recoveries from the spiked pesticide samples were based on unspiked control samples. The pesticide recoveries ranged from 85.2 to 118%. Recoveries for the Aroclor samples based on five major peaks ranged from 75 to 83%. For further verification the PCB samples were spotted on thin layer plates and compared with gas chromatographic data. The TLC recoveries ranged from 75 to 90%. Residues were not corrected for percent recovery.

All samples were placed on two different columns for electron capture detection and then concentrated to 1 ml or below for analyses on the Coulson detector.

Results

The percent of human milk samples with residues of organochlorine pesticides and PCB's are presented in Table 1. *P,p'*-DDE and *p,p'*-DDT were found in all 40 milk samples. β-BHC was found in 87.5% and PCB in 20% of the samples.

TABLE 1.—Percent of total milk samples with organochlorine pesticide residues and PCB's, based on 40 samples, Colorado—1971-72

COMPOUND	PERCENT POSITIVE SAMPLES
<i>p,p'</i> -DDE	100.00
<i>p,p'</i> -DDT	100.00
β-BHC	87.5
<i>o,p'</i> -DDT	72.5
<i>p,p'</i> -DDD	35.0
Dieldrin	45.0
Heptachlor epoxide	25.0
PCB's	20.0

The levels of selected organochlorine pesticides and PCB's in each of the 40 milk samples are listed in Table 2. The highest levels found were of *p,p'*-DDE which ranged from 19 to 386 ppb and *p,p'*-DDT, from 7 to 09 ppb. β -BHC, *o,p'*-DDT, dieldrin, heptachlor epoxide, and *p,p'*-DDD ranged from a trace to 38, 13, 11, 5, and 5 ppb, respectively. PCB's ranged from 40 to 100 ppb.

Values for total organochlorine pesticides and PCB's in each sample are given in Table 3 with selected epidemiological data for each donor: age, occupation, birthplace, number of years lived in Colorado, and whether the participant had ever lived in a city of 50,000 population or greater. The total organochlorine levels ranged from a high of 521 to a low of 27 ppb. The age of participants ranged from 19 to 33 years.

Discussion

All samples contained *p,p'*-DDE and *p,p'*-DDT; β -BHC was found in 87.5%, *o,p'*-DDT in 72.5%, dieldrin in 45%, and PCB's in 20% of the samples. Milk from one study participant (No. 20) contained quantifiable levels of all compounds except heptachlor epoxide. Heptachlor epoxide and PCB's were never found in the same sample in the study, and *p,p'*-DDD and PCB's were found in the same sample on only one occasion. The total organochlorines per sample ranged from 27 to 521 ppb. The sample with the lowest level of organochlorines contained *p,p'*-DDE and *p,p'*-DDT. The sample with the highest level contained β -BHC, *p,p'*-DDE, *p,p'*-DDT, dieldrin, and a trace of *o,p'*-DDT, *p,p'*-DDD, and heptachlor epoxide. The highest level of total organochlorines occurred in the sample from a 24-year-old housewife who had spent all but 1 year of her life in Colorado. Only nine women had not lived in a city of 50,000 population or greater. Two of these nine were positive for PCB's. The fact that *p,p'*-DDE and *p,p'*-DDT were found in all samples was not surprising as Laug *et al.* (5) found DDT in 30 of 32 milk samples collected in the Washington, D. C., area in 1951.

Newton and Greene (7) found DDT and DDE in all of 67 human milk samples collected in Australia. Dieldrin was recovered in approximately 43% of the Australian

samples and in 45% of the samples in our study. Dymant *et al.* (2) did not find PCB's in human milk collected from residents of New Guinea and Texas, but Finklea *et al.* (3) in a study of plasma collected from volunteers living in Charleston County, S. C., found PCB's in 43% of the study participants. In the current study, we found PCB's in 20% of the samples.

Results of this study and other studies indicate that organochlorines are widespread in human milk. Although *p,p'*-DDE and *p,p'*-DDT are found almost universally, *p,p'*-DDD, dieldrin, heptachlor epoxide, and PCB's are not as widely distributed.

See Appendix for chemical names of compounds discussed in this paper.

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TABLE 2.—Residues of selected organochlorine pesticides and PCB's in 40 human milk samples, Colorado—1971-72

DONOR NUMBER	RESIDUES IN PPB (fat basis)							
	β -BHC	<i>p,p'</i> -DDE	<i>o,p'</i> -DDT	<i>p,p'</i> -DDT	<i>p,p'</i> -DDD	DIELDRIN	HEPTACHLOR EPOXIDE	PCB's
1	5	29	13	16	5	—	—	—
2	3	98	—	25	—	—	—	—
3	—	19	—	8	—	—	—	—
4	9	123	—	20	—	—	—	—
5	6	36	—	21	—	—	—	—
6	3	30	8	12	—	—	—	—
7	3	52	6	22	T	T	—	—
8	5	82	7	21	—	T	—	—
9	7	138	12	32	T	3	5	—
10	6	72	4	13	T	T	2	—
11	5	79	3	14	T	9	T	—
12	5	31	6	15	T	4	2	—
13	4	24	5	8	T	—	2	—
14	2	37	3	11	—	—	T	—
15	—	57	—	13	—	T	—	—
16	2	46	—	13	—	T	—	—
17	2	79	5	15	4	T	—	—
18	6	37	2	7	—	—	—	—
19	5	49	3	8	—	—	—	—
20	14	166	7	27	3	5	—	100
21	11	135	7	24	3	10	T	—
22	5	68	5	10	T	4	4	—
23	6	104	6	26	3	—	T	—
24	15	386	1	109	T	11	T	—
25	9	90	3	12	1	—	—	—
26	T	59	—	13	—	—	—	—
27	T	30	—	14	—	8	—	—
28	1	83	4	14	—	—	—	—
29	6	51	10	16	—	4	—	40
30		158	8	31	—	—	—	—
31		75	6	13	—	—	—	40
32		42	1	16	—	3	—	50
33	38	192	10	42	—	—	—	40
34	7	84		20	—	4	—	—
35	7	186	10	39	—	—	—	40
36	10	98	10	24	—	—	—	40
37	1	41	—	10	—	—	—	40
38	6	58	—	11	—	—	—	—
39	3	124	4	22	—	—	—	—
40	22	262	11	43	—	4	—	—
Range	T-38	19-386	T-13	7-109	T-5	T-11	T-5	40-100

NOTE:— = not detected; T = trace (present at concentrations below the sensitivity level, confirmed qualitatively but not quantifiable).

TABLE 3.—Total organochlorine and PCB's in milk samples, ranked from highest to lowest, in selected epidemiological data for sample donors, Colorado—1971-72

RANK OF SAMPLES	DONOR NUMBER	AGE (YEARS)	OCCUPATION	BIRTHPLACE	NUMBER OF YEARS LIVED IN COLORADO	LIVED IN CITY WITH POPULATION OF 50,000	TOTAL ORGANOCHLORINES AND PCB'S (PPB)
1	24	24	Housewife	Colorado	23	Yes	521
2	40	29	Housewife	Florida	8	Yes	342
3	20	25	Housewife	Colorado	25	Yes	322
4	33	23	Housewife	Japan	1	Yes	322
5	35	19	Housewife	North Dakota	7 mos	No	282
6	9	28	Nurse	New York	2.5	Yes	197
7	30	27	Housewife	Colorado	2	Yes	197
8	21	27	Housewife	Minnesota	1	Yes	190
9	36	25	Secretary	Ohio	2	Yes	182
10	39	25	Medical technician	Hawai	4	Yes	153
11	4			unknown			152
12	23			unknown			145
13	31	26	Housewife	Michigan	22	No	134
14	29	22	Housewife	Germany	3	Yes	127
15	2	25	Housewife	Colorado	25	No	126
16	8	22	Housewife	Nebraska	3.5	Yes	115
17	25			unknown			115
18	34	32	Secretary	Kentucky	2.5	Yes	115
19	32	20	Housewife	Colorado	20	No	115
20	11	24	Housewife	Louisiana	8 mos.	Yes	110
21	17	28	Housewife	California	2.5	Yes	105
22	28	26	Nurse	Illinois	4	Yes	101
23	10	33	Housewife	Colorado	31	Yes	97
24	22	23	Housewife	Missouri	2.5	No	96
25	37	20	Housewife	Colorado	19	Yes	95
26	7	28	Student	Indiana	7 mos.	Yes	83
27	38	23	Housewife	Colorado	23	No	75
28	26	24	Housewife	Colorado	18	Yes	72
29	15	20	Beautician	Wyoming	19	No	70
30	1	30	Editor	Illinois	9	Yes	68
31	19	19	Housewife	South Dakota	12	Yes	65
32	5	22	Elementary teacher	Colorado	10	Yes	63
33	12	23	Housewife	Indiana	1	Yes	63
34	16	28	Housewife	Iowa	9 mos.	Yes	61
35	18	23	Housewife	Pennsylvania	10	Yes	53
36	6	29	Teacher	Kansas	1.5	Yes	53
37	14	30	Housewife	California	3	No	53
38	27	21	Beautician	Wyoming	7 mos.	No	52
39	13	29	Housewife	New York	1	Yes	43
40	3	29	Professor	Missouri	6	Yes	27

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

*Accumulation and Movement of Mirex in Selected Estuaries of South Carolina, 1969-71*¹

P. W. Borthwick², T. W. Duke², A. J. Wilson, Jr.², J. I. Lowe²,
J. M. Patrick, Jr.², and J. C. Oberheu³

ABSTRACT

In conjunction with a fire ant eradication program during which mirex was aerially applied to coastal areas near Charleston, S. C., field studies were conducted to monitor the movement and accumulation of mirex in the estuarine system.

Collections of background and periodic posttreatment samples of water, bottom sediments, shrimp, crabs, fish, and estuary-dependent birds and mammals were analyzed for mirex using electron-capture gas chromatography.

The data revealed that (1) mirex was translocated from treated lands and high marsh to estuarine biota—all animal classes sampled contained mirex; and (2) biological concentration of mirex occurred—especially in predators such as racoons and birds.

Mirex residue ranges for respective sample categories were: water (<10.01 ppb); sediment (0-0.07 ppm); crabs (0-0.60 ppm); fishes (0-0.82 ppm); shrimps (0-1.3 ppm); mammals (0-4.4 ppm); and birds (0-17.0 ppm). No mass mortalities were observed during the study.

Introduction

Mirex, a chlorinated hydrocarbon, is the insecticide component of a bait used in the Southeastern United States to control the imported fire ant (*Solenopsis saevissima richteri* Forel). This bait was developed after various pesticides and pesticide-bait formulations applied to control the ants proved to be toxic to nontarget or-

ganisms. Large-scale applications of dieldrin and heptachlor were especially destructive to fish and wildlife (2). Mirex was developed specifically to control fire ants and, until recently, was not considered to be toxic to nontarget organisms.

Independent experiments conducted under controlled conditions in the laboratory at Gulf Breeze, Fla., and at Bears Bluff, S. C., showed this chemical to be toxic to decapod crustaceans, including juvenile blue crabs and penaeid shrimp (1, 5, 6). Because of these and other results and concern of commercial fishermen that application of mirex to marsh areas could adversely affect fishery resources, application of mirex to the coastal environment was suspended and this cooperative study was undertaken.

The Gulf Breeze Laboratory (formerly a biological laboratory of the Bureau of Commercial Fisheries, Fish and Wildlife Service, U.S. Department of the Interior) entered an agreement with the U.S. Department of Agriculture in September 1969, to study the accumulation and movement of mirex in the estuarine environment near Charleston, S. C. The Bureau of Sport Fisheries and Wildlife of the Fish and Wildlife Service also agreed to participate in the study which terminated July 1, 1971.

The Gulf Breeze Laboratory was responsible for designing the study; collecting samples of bottom sediment, water, shrimp, crabs, and fish; and for analyzing all samples. The Bureau of Sport Fisheries and Wildlife was responsible for collecting birds and mammals.

The purposes of this investigation were (1) to observe the possible movement of mirex from treated areas near

¹Contribution No. 156 from the Gulf Breeze Environmental Research Laboratory, U.S. Environmental Protection Agency, Gulf Breeze, Fla. 32561, an Associate Laboratory of the National Environmental Research Center, Corvallis, Oreg.

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Charleston, S. C., to the estuarine environment and (2) to determine levels of mirex in organisms, particularly crabs and shrimp, before, during, and after treatment of the area. The investigation began approximately 1 week before the first treatment was applied. Pretreatment samples were collected to establish "background" levels of mirex in the environment. The short time period between the start of the investigation and the application of mirex, however, precluded studies necessary to determine the ecological impact of this chemical on the study area.

Methods

STUDY AREA

The estuaries in which these studies were conducted were located on a fire ant treatment area that extended 30 miles on either side of a line from Columbia to Charles-

ton, S. C. The boundary of the treatment area ended approximately 12 miles from the coast (Fig. 1). Mirex, therefore, was not applied directly to the salt marsh, except in an experimental plot near Toogoodoo Creek.

The topography and biota of the estuarine environment are unique and often present special problems to environmental studies. Estuaries along this portion of the East Coast of the United States are protected by barrier islands and are supplied silt-laden fresh water by creeks and rivers on the mainland. These typical "Spartina" marshes support transient populations of crabs, shrimp, and fish that develop to maturity in the estuary, then return to the sea. In addition, resident populations of shellfish and some fin-fish inhabit the estuary throughout their lives. Many predatory birds and mammals depend upon the estuarine organisms for food.

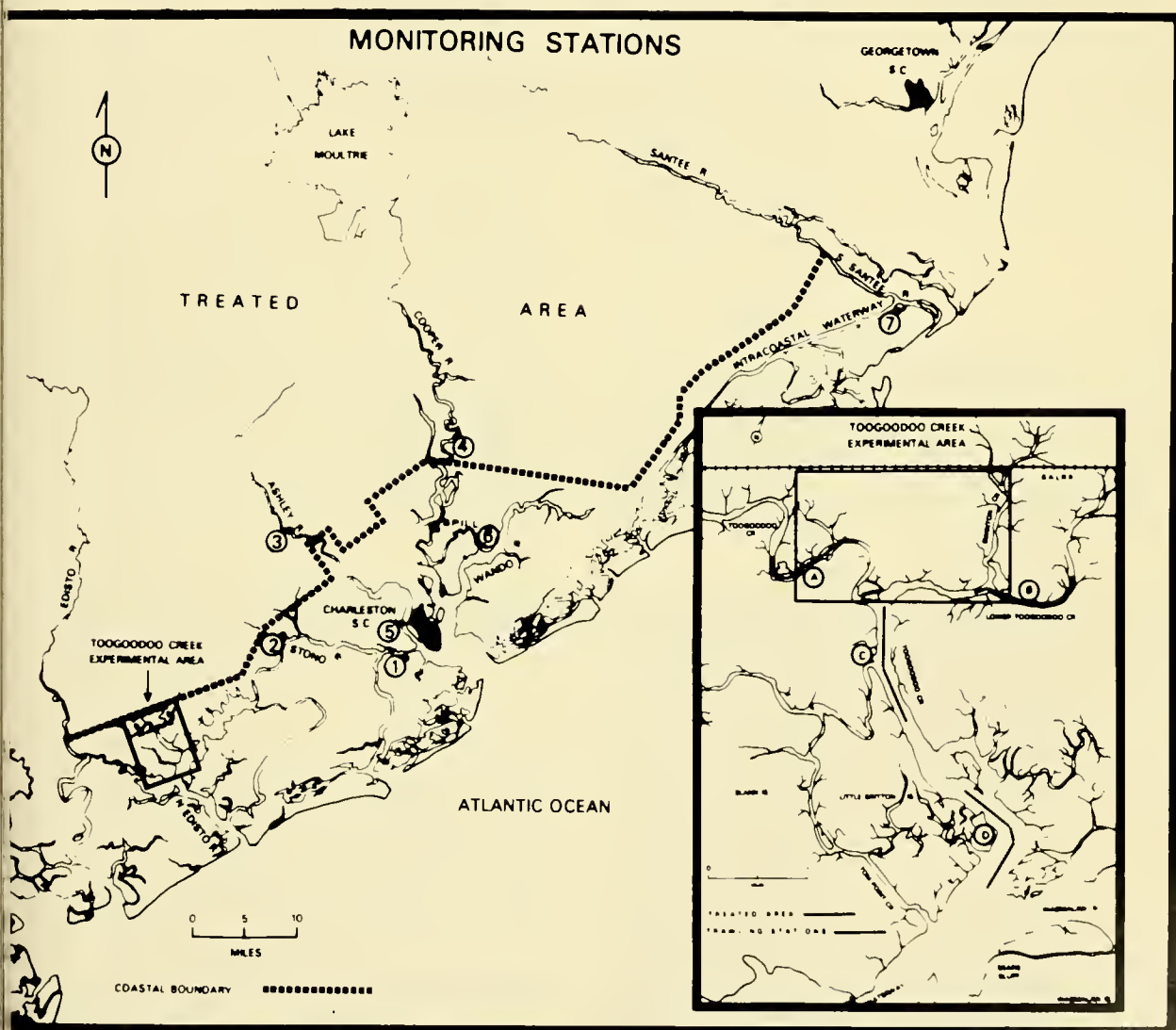


FIGURE 1.—Sampling sites in selected estuaries of South Carolina, 1969-71

Descriptions of sampling sites with the dates of mirex applications are given in Table 1.

Periodically, levels of mirex in water, sediment, and biota were monitored at (1) four stations near the main inland mirex-treated area and within and near a 2-square mile plot of salt marsh that was treated experimentally, (2) six stations located on the major rivers that drain the main inland mirex-treated areas, and (3) a station in a semi-enclosed tidal pond 4 miles from the main inland

mirex-treated area; the banks of the pond (2.5 acres) were treated with mirex by hand spreader.

APPLICATION OF MIREX

The mirex 4X Bait formulation contained 84.7% corn-cob grits, 15.0% soybean oil, and 0.3% mirex. The bait was applied at a rate of 1.25 lb per acre or 1.7 g of technical mirex. This produced approximately 16 particles of bait per square foot.

TABLE 1.—Description of sampling sites and dates of mirex application, South Carolina, 1969-71

MAP LOCATION NUMBER (FIG. 1)	NAME AND LOCATION OF SAMPLING SITE	REMARKS	APPLICATION DATES		
			1ST	2d	3d
A	Toogoodoo Creek Lat. 32° 41' N Long. 80° 18' W	Within the main inland mirex-treated area on the upper reaches of the left branch of the Creek. Tidal marshlands predominate along the north bank; pine woodlands lie to the south. Several homes are in the area. Also treated experimentally by helicopter.	10/14-15/69	6/3-4/70	10/27-28/70
B	do.	Begins at the mouth of Swinton Creek and continues eastward on lower part of the Creek; partially within the mainland treated zone; marsh and woodland areas are on each bank. A few homes are along the south bank. Also treated experimentally by helicopter.	do.	do.	do.
C	do.	Extends southward from the fork of Toogoodoo Creek, just south of the experimental area treated by helicopter. Extensive farmlands are on the west bank, tidal marshlands on the east.	do.	do.	do.
D	do.	Begins 2 miles south of the helicopter-treated area and continues to the mouth of Toogoodoo Creek on the Intracoastal Waterway. Uninhabited low marsh areas on both sides of the creek are riddled with many small tidal creeks.	do.	do.	do.
1	Riverland Terrace Pond Lat. 32° 46' N Long. 79° 59' W	Seven miles from main inland treatment area, includes one of two adjoining 1-acre ponds in Riverland Terrace (a residential area). The east pond floods and drains with the tides into Wappoo Creek (Intracoastal Waterway), via two large culverts. The banks of the east pond were treated above the high tide mark by hand spreader.	12/3/69	7/24/70	12/1/70
2	Stono River (Intracoastal Waterway) at Log Bridge Creek Lat. 32° 45' N Long. 80° 08' W	Two miles from main inland treatment area. Several homes along the east side of the creek; the west side is bordered by tidal marshes.	10/23/69	6/18/70	—
3	Ashley River at Runnymede Plantation Lat. 32° 53' N Long. 80° 05' W	Old plantations and rural homes are located on the east bank, and tidal marshes lie along the west side. This fresh water station is inside the main inland mirex-treated area.	10/22/69	6/11/70	—
4	Cooper River at U.S. Naval Ammunition Depot Lat. 32° 57' N Long. 79° 56' W	Bordered on the west bank by high wooded ground. On the east side, tidal marshes predominate. This fresh water station is inside the main inland mirex-treated area.	10/17/69	6/10/70	—
5	Ashley River at Oldtown Creek Lat. 32° 48' N Long. 79° 58' W	Six miles from the main inland treated area. The Citadel Military College is on the east bank, Charles Towne Landing on the west. Several industrial plants and homes, in addition to tidal marshes, are on this portion of the river.	10/22/69	6/10/70	—
6	Wando River at Beresford Creek Lat. 32° 53' N Long. 79° 53' W	Six miles from the main inland treated area. This uninhabited area is bordered by expansive marshlands and marsh islands.	10/17/69	6/8/70	—
7	South Santee River (Intracoastal Waterway) at Alligator Creek Lat. 32° 08' N Long. 79° 19' W	Six miles from the main inland treated area. This location is uninhabited and near the Cape Romain Migratory Bird Refuge.	9/18/69	5/20/70	—

Airex 4X Bait was applied by fixed wing multi-engine aircraft (PV-2) to the inland treatment area, by helicopter to the Toogoodoo experimental plot, and by hand spreaders to the banks of a tidal pond. All applications were supervised by USDA.

The inland treatment area was divided into blocks that varied from 350,000 to 450,000 acres. An electronic guidance system (Decca Survey System) was set up in each block. This system consisted of three transmitting and receiving stations (one master station and two slave stations). The master and one of the slave stations produced an electronic tracking signal. Equipment mounted in the application aircraft received this signal which was fed through a computer into a dacometer. By use of the dacometer, the aircraft pilot could follow the signal from one point to another along the tracking path. Uniform application was made across each block by flying along a series of parallel signals. The master station and the second slave station produced a ranging signal that designated the points along the tracking path where the spraying was cut off. These signals, tracking and ranging, were converted to a numbering system and the system oriented to a map of the area being treated.

To allow for better control, helicopter applications of mirex bait at the recommended rate were made to 100 acres of the 1,200-acre Toogoodoo Experimental Area. The Bell G-2-A helicopter used for spraying was equipped with two side-mounted hoppers (300 pounds capacity each) with electrically operated hopper gates, agitators, spinner-vane wheels, and spreaders for "positive control" at the "cut-off point." Flying at an airspeed of 40 miles per hour and an altitude of 80 feet produced a 75-foot wide swath; at 40 feet, a 60-foot wide swath. Helium-filled kytoons were used to mark each swath path. Bait distribution was even at both altitudes, but wind caused considerable aerial drift.

Approximately 2.5 acres surrounding the tidal pond were treated three times at 6-month intervals by hand-operated seed spreaders.

SAMPLING

Materials Sampled

Pesticides entering the estuarine environment become part of the biogeochemical cycles continually in operation within that environment. Therefore, the water, sediment, and various biota were sampled to determine routes of movement and reservoirs of the chemical in the study area. Common and scientific names of aquatic animals, birds, and mammals collected for mirex analysis are listed in Table 2. Crabs and shrimp were of special interest because of their sensitivity to mirex. Bottom and filter-feeding fish were also collected since they could accumulate mirex from food web organisms

that were often difficult to obtain because of their transitory nature and movement to deeper water during the winter. In general, representatives of all species of aquatic organisms caught in the trawl were analyzed for mirex.

In selecting the birds and mammals for residue analysis, an effort was made to pick species that were (a) directly dependent on the estuarine environment for food; (b) most likely to live and feed in the area where they were collected; and (c) plentiful enough to allow periodic collections with a minimum of difficulty.

The raccoon was selected as the mammal for sampling since it is the most abundant carnivore in the salt marsh, where it preys heavily upon crustaceans and shellfish. It was more difficult to decide upon a bird species. Numerous gulls, shore birds, wading birds, and other water birds are found in the estuary, but most of them are migratory by nature, moving up and down the coast in search of food or as a result of weather changes. Few water birds are year-round residents. The clapper rail was finally selected as the most sedentary and widely distributed bird in the estuaries: when rails were not available at a particular sampling station, wading birds were collected. All animals collected during this study are listed in Table 2.

Collection of samples

Aquatic animals were collected with a 12-foot, $\frac{3}{4}$ -inch bar mesh, otter trawl towed at 3 to 6 knots for 20 minutes at each station. Pond collections were made with a 15-foot, $\frac{1}{4}$ -inch mesh haul seine. Occasionally, animals near the surface were taken with dip nets; fiddler crabs and oysters were collected by hand. Raccoons were captured with wire live-traps; birds were hunted on foot or from a boat.

Several methods for collecting water (including carbon filtration) were considered, but because of the high silt content of the well-mixed water, water was collected just below the surface in a 1-gallon glass jug and sealed with a teflon-lined cap. A modified grab sampler was used to collect bottom sediment at each station. A sampler especially designed to collect the upper few centimeters of bottom sediment was used in specified instances.

Frequency of sampling

A pretreatment (background) and numerous posttreatment samples were taken at all stations. Quarterly collections of birds and mammals were scheduled around the first week in September, December, March, and June. Periodically, this schedule was altered by 2 or 3 weeks to select the week of highest tides for the best

TABLE 2.—Common and scientific names of aquatic animals, birds, and mammals collected for mirex residue analysis

CRABS		FISHES—Continued	
Blue crab	<i>Callinectes sapidus</i> Rathbun	Star drum	<i>Stellifer lanceolatus</i> (Holbrook)
Common mud crab	<i>Panopeus herbstii</i> H. Milne Edwards	Striped killifish	<i>Fundulus majalis</i> (Walbaum)
Mud crab	<i>Rithropanopeus harrisi</i> Gould	Striped mullet	<i>Mugil cephalus</i> Linnaeus
Portunid crab	<i>Callinectes ornatus</i> Ordway	Weakfish	<i>Cynoscion regalis</i> (Bloch and Schneider)
Sand hiddler	<i>Uca pugnator</i> (Bosc)	White catfish	<i>Ictalurus catus</i> (Linnaeus)
SHRIMPS		White mullet	<i>Mugil curema</i> Valenciennes
Brown shrimp	<i>Penaeus aztecus</i> Ives	Winter flounder	<i>Pseudopleuronectes americanus</i> (Walbaum)
Brown-spotted shrimp	<i>Penaeus duorarum</i> Burkenroad	MISCELLANEOUS AQUATIC ANIMALS	
Grass shrimp	<i>Palaeomonetes pugio</i> Holthuis	American oyster	<i>Crassostrea virginica</i> (Gmelin)
River shrimp	<i>Macrobrachium ohione</i> (Smith)	Brief squid	<i>Lolliguncula brevis</i> (Blainville)
White shrimp	<i>Penaeus setiferus</i> (Linnaeus)	Nudibranch	<i>Doris</i> sp.
FISHES		Southern periwinkle	<i>Littorina irrorata</i> (Say)
American eel	<i>Anguilla rostrata</i> (Lesueur)	BIRDS	
Atlantic croaker	<i>Micropogon undulatus</i> (Linnaeus)	American bittern	<i>Botaurus lentiginosus</i> (Rackett)
Atlantic menhaden	<i>Brevoortia tyrannus</i> (Latrobe)	American egret	<i>Casmerodius albus</i> (Linnaeus)
Atlantic silverside	<i>Menidia menidia</i> (Linnaeus)	Anhinga	<i>Anhinga anhinga</i> (Linnaeus)
Atlantic thread herring	<i>Opisthonema oglinum</i> (Lesueur)	Belted kingfisher	<i>Megasceryle alcyon</i> (Linnaeus)
Bay anchovy	<i>Anchoa mitchilli</i> (Valenciennes)	Clapper rail	<i>Rallus longirostris</i> Boddgert
Blackcheek tonguefish	<i>Symphurus plagiusa</i> (Linnaeus)	Common snipe	<i>Capella gallinago</i> (Linnaeus)
Black drum	<i>Pogonias cromis</i> (Linnaeus)	Green heron	<i>Butorides virescens</i> (Linnaeus)
Black sea bass	<i>Centropomus striata</i> (Linnaeus)	Least bittern	<i>Ixobrychus exilis</i> (Gmelin)
Bluefish	<i>Pomatomus saltatrix</i> (Linnaeus)	Little blue heron	<i>Florida caerulea</i> (Linnaeus)
Fourspot flounder	<i>Paralichthys oblongus</i> (Mitchill)	Louisiana heron	<i>Hydranassa tricolor</i> (Müller)
Hogchoker	<i>Trinectes maculatus</i> (Bloch and Schneider)	Marsh hawk	<i>Circus cyaneus</i> (Linnaeus)
Mummichog	<i>Fundulus heteroclitus</i> (Linnaeus)	Pied-billed grebe	<i>Podilymbus podiceps</i> (Linnaeus)
Pinfish	<i>Lagodon rhomboides</i> (Linnaeus)	Plover	<i>Charadrius</i> sp.
Sailfin molly	<i>Poecilia latipinna</i> (Lesueur)	Snowy egret	<i>Leucophoyx thula</i> (Molina)
Sea catfish	<i>Arius felis</i> (Linnaeus)	Sora rail	<i>Porzana carolina</i> (Linnaeus)
Searobin	<i>Prionotus</i> sp.	Virginia rail	<i>Rallus limicola</i> Vieillot
Sheepshead	<i>Archosargus probatocephalus</i> (Walbaum)	White ibis	<i>Eudocimus albus</i> (Linnaeus)
Silver perch	<i>Bairdiella chrysura</i> Lacépède	Willet	<i>Catoptrophorus semipalmatus</i> (Gmelin)
Snook	<i>Centropomus undecimalis</i> (Bloch)	Yellow-crowned night heron	<i>Nyctanassa violacea</i> (Linnaeus)
Southern kingfish	<i>Menticirrhus americanus</i> (Linnaeus)	MAMMALS	
Spot	<i>Leiostomus xanthurus</i> Lacépède	Opossum	<i>Didelphis virginiana</i> (Linnaeus)
Spotted hake	<i>Urophycis regius</i> (Walbaum)	Raccoon	<i>Procyon lotor</i> (Linnaeus)
Spotted seatrout	<i>Cynoscion nebulosus</i> (Cuvier and Valenciennes)		

rail hunting. Samples were taken at the six river stations 24 hours and 3 months after each of two applications of mirex to the inland treatment area by fixed wing aircraft. Biweekly collections were made at the four stations in the Toogoodoo Creek Plot during the entire 18 months of the study; mirex was applied to the experimental plot by helicopter three times at 6-month intervals to high marsh only, i.e., marsh not normally covered by tidal waters. Samples were taken from the tidal pond 24 hours after each of three hand-spread treatments and at irregular intervals between applications.

ANALYTICAL PROCEDURES

Preparation of samples

Crabs, shrimp, and fish were prepared separately by pooling whole individuals, but birds and mammals were

prepared individually. Muscle tissue from breast and upper wing in birds and thigh in raccoons and oil glands and eggs from birds were analyzed. All samples were ground and mixed thoroughly in a blender. A 30-g subsample was blended with a desiccant mix composed of 10% QUSO (a microfine precipitated silica) and 90% anhydrous sodium sulfate. This mixture was alternately frozen and blended until a free-flowing powder was obtained.

Sediment samples were spread on sheets of aluminum foil and dried at room temperature. The dry sediment was pulverized to a fine powder in a blender.

At this stage of preparation, the samples were wrapped in aluminum foil, packaged in plastic bags, and mailed to the Gulf Breeze Laboratory for extracting and pesticide residue analysis.

Water samples were refrigerated for up to 2 weeks until they were mailed to Gulf Breeze for analysis.

Analysis of samples

Tissues of shrimp, crabs, and fish mixed with the desiccant were extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated to approximately 10 ml and transferred in 3- to 4-ml portions to a 400-mm by 20-mm chromatographic column that contained 76 mm of unactivated Florisil. After each portion settled in the column, vacuum was applied to evaporate the solvent. This was repeated after each addition and after three 5-ml petroleum ether rinses of the extraction flask. The vacuum pump was disconnected after all solvent had evaporated, and the residue was eluted from the column with 70 ml of 9:1 mixture of acetonitrile and distilled water. The eluate was evaporated to dryness and the residue transferred to a Florisil column (7) with petroleum ether.

Sediment samples were dried at room temperature and extracted for 4 hours with 10% acetone in petroleum ether in a Soxhlet apparatus. Extracts were concentrated to approximately 10 ml and transferred to a Florisil column (7).

Water samples were not filtered before being extracted with petroleum ether. The extracts were dried with anhydrous sodium sulfate and reduced to an appropriate volume.

The extracts of all substrates were identified and measured by electron capture gas chromatography. Extract volumes were adjusted to obtain a sensitivity of 0.01 ppm (mg/kg) for tissue and sediment samples and 0.01 ppb ($\mu\text{g}/\text{liter}$) for water samples. Operating conditions of the two 152.4-cm by 3.2-mm glass columns used were:

Liquid phase:	2% OV-101	1:1 2% OV-101
Solid Support:	100/120 Gas Chrom Q	100/120 Gas Chrom Q
Temperatures:		
Oven:	188° C	180° C
Injector and detector:	210° C	210° C
N ₂ flow rate:	25 ml/min	25 ml/min

Laboratory tests indicated recovery rates for mirex were greater than 85%. Data in this report do not include a correction factor for percent recovery. All residues reported are on a wet-weight basis, except those of sediments, which are reported on a dry-weight basis. Thin layer chromatography, "p" values, and mass spectrometry were used to confirm the presence of mirex.

STATISTICAL ANALYSIS OF DATA

All statistical comparisons were made with the χ^2 -test for independent samples (8), and differences were consid-

ered real at the 0.01 level of significance. The movement of mirex into the aquatic environment and its consequent accumulation in populations of estuarine animals were presumed to be greatest in areas where a significantly greater proportion of samples was positive for mirex (>0.01 ppm).

Average residues reported were computed by assuming that samples where mirex was not detected (<0.01 ppm) actually had no residue.

Bird and mammal residue data were not analyzed statistically; however, average mirex residues in muscle tissues are tabulated for herons and egrets (Table 10), clapper rails (Table 11), and raccoons (Table 13).

Results

PRETREATMENT SAMPLES

Mirex was not detected in any pretreatment samples of crabs, shrimp, fish, sediment, or water taken from the six monitoring stations, Toogoodoo stations, or the Riverland Terrace pond. Mirex residues were found in about one-third of the pretreatment samples of migratory birds (Table 9). Only one raccoon was collected before treatment, and this sample was free of mirex residues. Background residues in birds collected in the Charleston area are discussed in the section on "Significance of Data."

WATER AND SEDIMENT SAMPLES

Mirex was not detected in water samples during the study. No attempt was made, however, to concentrate water samples (such as carbon filtration of large volumes of water).

Sediment samples were negative, except in six instances (three samples from Riverland Terrace pond and three from the Ashley River within the treated zone). Accordingly, mirex residues in water and sediment are not reported in tabular form.

ACCUMULATION OF MIREX IN BIOTA

Biological concentration of mirex occurred in the estuarine food web as shown below:

SAMPLE	RESIDUE RANGE (ppm)	PERCENT OF POSTTREATMENT SAMPLES WITH MIREX RESIDUES
Water	<0.01 ppb	0
Sediment	0*-0.07	3
Crabs	0-0.60	31
Fishes	0-0.82	15
Shrimps	0-1.3	10
Mammals	0-4.4	54
Birds	0-17.0	78

*0 = <0.01 ppm

Differences in the amounts of mirex accumulated by the animals were probably caused by variables such as proximity to the treated area, duration of exposure to a

mirex-contaminated habitat, seasonal habits, avoidance ability, and position in the food web.

Additional variation may depend upon parameters such as method of application, amount and frequency of rainfall, surface runoff, variations in sea level, and degradation.

Levels of mirex found in the biota are listed in Tables 5 to 14.

Discussion

ANIMAL MORTALITIES

Procedures used in this study were neither capable of, nor intended to comprehensively detect mortality of aquatic organisms. The sampling areas were, however, inspected for dead or affected animals during each sampling period, and mass mortalities would probably have been detected visually or in trawl catches. No mass mortalities of organisms were observed during the study. Our laboratory experiments (5) suggest that mortalities in a population of marine crustaceans due to mirex would not all occur at the same time. Symptoms of mirex poisoning exhibited by shrimp and crabs prior to death are irritability, uncoordinated movement, loss of equilibrium, and paralysis. An affected crab may live several days or even weeks after the initial exposure to mirex. Animals in advanced stages of poisoning would be highly susceptible to predation by larger carnivores and could be swept out of estuaries by tidal action. Thus, affected animals could be removed from the system without leaving visible evidence of their condition. Further, any dead animals would generally enter the detritus pool soon after death.

TOOGOODOO CREEK EXPERIMENTAL AREA—

1,200-acre plot (Fig. 1) treated by helicopter on Oct. 14-15, 1969; June 3-4, 1970; and Oct. 27-28, 1970

Movement of mirex from treated lands above the high-tide mark undoubtedly occurred after each of the three treatments, especially the first. The mechanisms of transporting mirex from treated land areas to the Toogoodoo Creek estuary are poorly understood. Surface runoff into the drainage system (especially after heavy rain) is one suspected cause.

All species sampled contained mirex. Residues first appeared in a shrimp sample 2 weeks after the first treatment. From then on, the relative frequency of mirex-positive samples and the "average" levels of mirex residues fluctuated greatly. Mirex was present, however, in at least one sample in 33 of 44 collections. Some of the statistically significant relationships occurring within these fluctuations are discussed in the following paragraphs.

Application effects

The relative number of samples that contained mirex appeared to increase during the first 10 weeks after each treatment and then to decrease in the 10- to 20-week period (Table 3). After the first and third treatments, the number of positive samples appeared to decrease even further in the third interval, 20 to 32 weeks.

These decreases for each individual treatment were not statistically significant; however, when data from all treatments were evaluated together, the relationship between time elapsed since spraying and the decreasing number of positive samples proved to be real.

Decreases in the percent occurrence of mirex residues from the first to the third treatment were significant. This could be due to: (1) mirex being translocated rapidly from the estuarine biota to reservoirs in fatty tissues of predacious birds and mammals, (2) transient estuarine animals (e.g., crabs, shrimp) translocating mirex during emigration from nursery areas of Toogoodoo Creek, (3) possible differences in the manner in which mirex bait was applied by the two helicopter pilots, or (4) degradation of mirex by physical, chemical, or biological processes.

Location effects

The relative number of samples positive for mirex also fluctuated with station location. Stations A and B were located within the treated area, and Stations C and D were downstream from the treated area (Fig. 1). Mirex levels at these stations gave some indication of the movement of mirex from treated land areas into untreated areas downstream.

As expected, more animals from the treated area contained mirex than did those from downstream stations (Table 4). This relationship was statistically significant after the first and third applications, but was only apparent after the second treatment. The frequencies of positive samples at stations located within the treated zone (A and B) were not significantly different. As shown in Table 4, there was an apparent decrease in frequency of positive samples with increased distance from the treated zone.

Species effects

The percent occurrence of mirex was higher in crab samples than in fish or shrimp samples. Although this difference was apparent at Stations A through D, it was statistically significant only at Station B. Overall, however, the higher residues in crabs were significant after the first two helicopter treatments, but was not statistically significant after the third treatment. The frequency of positive samples appeared unrelated to size of crabs or to species of fish.

TABLE 3.—Percent occurrence of mirex in crab, shrimp, and fish samples by time of sample collection in respect to mirex application at Toogoodoo Creek Experimental Area

APPLICATION	PERCENT OCCURRENCE OF MIREX BY SAMPLING TIME IN WEEKS SINCE APPLICATION			
	0-10	10-20	20-32	TOTAL WEEKS
First (Oct. 14-15, 1969)	37	36	18	30 (0-32 weeks)
Second (June 3-4, 1970)	22	9		16 (0-20 weeks)
Third (Oct. 27-28, 1970)	28	12	2	12 (0-32 weeks)
Overall Percent Occurrence	28	17	8	19

TABLE 4.—Percent occurrence of mirex in aquatic animals by sampling site with respect to treated area

SAMPLING SITE	LOCATION WITH RESPECT TO TREATED AREA	PERCENT OCCURRENCE MIREX IN ALL POST-TREATMENT SAMPLES OF CRABS, SHRIMP, AND FISH
A	Inside treated area	29%
B	Inside treated area	24%
C	Just outside treated area	17%
D	2 miles downstream from treated area	8%
3	Upstream stations located inside main inland treated area	70%
4		70%
2	Downstream stations located outside main inland treated area	30%
5		0%
6		25%
7		0%

MONITORING STATIONS—mirex was applied to inland treatment areas (Fig. 1) by fixed-wing aircraft during September-October 1969 and May-June 1970

Trends in the data were similar to those observed in the Toogoodoo Creek Experimental Area. The greatest number of positive samples occurred shortly after treatment and diminished with time. Samples positive for mirex were significantly more frequent within the treated area, upstream Stations 3 and 4, than at sites located outside the treated area, downstream Stations 2, 5, 6, and 7 (Table 4).

Although significant differences in percent occurrence were noted between these two groups, individual stations showed no significant differences because too few samples were taken and too few positive samples occurred for χ^2 -analysis. Also, for the individual monitoring stations, the relative number of samples containing mirex did not vary depending on the type of animal sampled.

A more frequent occurrence of mirex residues was apparent after the second application than after the first, but this increase was not significant.

RIVERLAND TERRACE POND—a 2.5-acre zone around the pond treated by hand-operated seed spreader on Dec. 3, 1969; July 24, 1970; and Dec. 1, 1970

Although mirex was applied three times to the banks of the pond, it was obviously not accumulated by sampled biota. During the study, only two crab samples contained mirex. In addition, three sediment samples were positive. The method of treatment (mirex applied by hand to a narrow bank around the pond above the high-tide mark on hard mud banks) might have caused the occurrence of the mirex in the sediment samples. "Crab samples" of sediment often consisted of as many as 20 to 25 "grabs" taken near the edge of the pond and one or more particles of bait could have been picked up with the sample.

Special attention was given Riverland Terrace Pond to observe any individual- or mass-mortalities in the pond biota. Migration of animals was controlled by means of retaining screens (¼-inch mesh) placed over two culverts that flood and drain the treated pond. Daily screen checks were made for a 3-week period to reveal any distressed, moribund, or dead animals. Crabs, shrimp, and fish observed in the pond or on the screens never appeared to be affected.

During several pond collections after treatment, seine-hauls revealed large populations of grass shrimp (mostly *Palaemonetes pugio*) and many of the females were gravid. On one occasion, grass shrimp were held for several weeks in an aquarium, where the shrimp remained healthy and their eggs seemed to develop and hatch normally. At no time was mirex detected in the grass shrimp population.

SIGNIFICANCE OF DATA

Surprisingly, mirex appeared in one-third of the birds in the pretreatment sample. Since the study area had not been previously treated with a large-scale application of mirex, the birds must have accumulated the residues from some other area. A large acreage around Savannah, Ga., had been treated with three successive applications of mirex in a pilot study of the feasibility of eradication. This area is the likely source of the mirex residues. Thus, migration is an important factor in interpreting the study data. Stewart's (9) report that northern clapper rails banded at Chincoteague, Va. migrated southward to winter in the coastal marshes of the South Atlantic States, including South Carolina, supports this view.

Measurable levels of mirex appeared at all stations, demonstrating that tidal flushing, biological transport, or some other mechanism can distribute the chemical throughout the estuary, regardless of precautions taken

to avoid treatment in the tidal zone. This finding is evidence that mirex can become widespread in animal food webs.

The occurrence and amount of mirex in birds and mammals varied considerably at all stations. This is to be expected since all of these animals are more or less migratory, and food sources of individuals vary. Similarly, Keith (4) found that levels of insecticide residues in fish-eating birds vary considerably within local populations of most species. Even so, average residues that appeared at the different stations correlated well with station location in respect to treated area, with the highest residues occurring at stations within a treated area and the lowest at stations farthest from the source of contamination.

Approximately 78% of the 179 birds collected after treatment began contained measurable residues of mirex, whereas residues were present in only 54% of the raccoons. The greater mobility of birds is doubtless the reason for this difference. In any case, occurrence of mirex residues as well as the quantity of residue in the animal appear directly related to drainage and distance from a treated area.

Residues in animals collected from the 2-square-mile treatment area on the Toogoodoo creek marshes (Stations A, B, and C) were not as great as those from animals collected within the inland treatment zone. This is not surprising because water as well as food-chain organisms in the Toogoodoo marshes were flushed twice daily by 4- to 6- foot tides.

Local and seasonal migrations of the sampled species would tend to mask any evidence of residue buildup during the course of the study. Even so, the absence of any individuals with greatly elevated residue levels indicates that average levels did not continue to rise beyond levels reached in the first few months following treatment. Raccoons were the most sedentary animals sampled. Data in Table 12 indicate that there was no gradual buildup of mirex residues in raccoons, although some seasonal variations are apparent.

The highest mirex residue (17.0 ppm) found in any animal analyzed occurred in a kingfisher. The highest level found in a raccoon was 4.40 ppm. All birds and mammals that contained residues in excess of 1.00 ppm are listed in Table 14. All animals on this list, except the kingfisher from Station D and two raccoons from Sta-

tion 2, came from stations classified as having a "high" mirex exposure potential.

See Appendix for chemical name of mirex

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TABLE 5.—*Mirex residues in shrimps by sampling site and sampling time, South Carolina, 1969-71*
[— = not detected]

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM—WHOLE BODY)	SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM—WHOLE BODY)
SAMPLING SITE A—TOOGOODOO CREEK					SAMPLING SITE C—TOOGOODOO CREEK				
White shrimp	Background	3	2.5	—	White shrimp	Background	3	5	—
First application (Oct. 14-15, 1969)					First application (Oct. 14-15, 1969)				
White shrimp	24 hrs	4	2.5	—	White shrimp	24 hrs	6	4-5	—
	2 wks	4	2.5	—		2 wks	4	3	—
	4 wks	10	4-5	.014		4 wks	10	4-5.5	.020
Brown shrimp	30 wks	13	2.5-3.5	—		6 wks	10	3.5-4	.014
Brown-spotted shrimp	32 wks	10	3.5-4	—	Brown-spotted shrimp	30 wks	1	5.5	—
Second application (June 3-4, 1970)						32 wks	3	6-7	—
Brown-spotted shrimp	24 hrs	12	3.5-5.5	.014	Second application (June 3-4, 1970)				
	2 wks	12	5-5.5	—	Brown-spotted shrimp	24 hrs.	9	4-5	.015
	4 wks	12	5.5-6	—		2 wks	12	3-5	—
	6 wks	12	5-6	.024		4 wks	12	4-6	—
	8 wks	12	3.5-5	—		6 wks	12	5-6	—
White shrimp	10 wks	12	3.5-4.5	—		8 wks	4	4	—
	12 wks	12	2.5-3.5	—	White shrimp	10 wks	1	4.5	—
	14 wks	12	3-4.5	—		12 wks	12	2.5-4	—
	16 wks	12	4-4.5	—		14 wks	12	4.5-5.5	—
	18 wks	11	5.5-6	—		16 wks	12	4-4.5	—
	20 wks	12	5-6	—		18 wks	12	5.5-6	—
Third application (Oct. 27-28, 1970)						20 wks	10	5-6	—
White shrimp	24 hrs	12	5-6	—	Third application (Oct. 27-28, 1970)				
	2 wks	4	5-6	—	White shrimp	24 hrs	12	5-6	—
Brown-spotted shrimp	20 wks	5	2.25-2.5	—		2 wks	10	5-7	—
	24 wks	5	2.5-3.5	—	Brown-spotted shrimp	20 wks	4	3.5-4.5	—
	28 wks	4	3.5-4.5	—	Grass shrimp	24 wks	132	.75-1.25	—
	30 wks	12	2.5-5	—	Brown-spotted shrimp	26 wks	9	3.5-5.5	—
	32 wks	12	2-4	—		28 wks	12	3.5-5	—
SAMPLING SITE B—TOOGOODOO CREEK						30 wks	12	4-5	—
White shrimp	Background	4	3	—		32 wks	12	3.5-5	—
First application (Oct. 14-15, 1969)					SAMPLING SITE D—TOOGOODOO CREEK				
White shrimp	24 hrs	4	3	—	White shrimp	Background	2	6	—
	2 wks	4	3-4	.040	First application (Oct. 14-15, 1969)				
	4 wks	10	4-5	.052	White shrimp	24 hrs	2	5	—
Brown-spotted shrimp	30 wks	2	3-5	—		2 wks	2	4.5	—
	32 wks	3	4	—		4 wks	7	5-6	—
Second application (June 3-4, 1970)						6 wks	10	4-5	—
Brown-spotted shrimp	24 hrs	12	5-6	—		8 wks	15	3.5-5	.027
	2 wks	12	5-5.5	—	Brown-spotted shrimp	24 wks	2	4.5-5	—
	4 wks	2	4-6	—		30 wks	2	3-3.5	—
	6 wks	5	4-7	—	Second application (June 3-4, 1970)				
	8 wks	12	4-5	—	Brown-spotted shrimp	24 hrs	12	4-5	—
White shrimp	10 wks	12	3-4	—		2 wks	12	5-6	—
	12 wks	12	2-3	—		4 wks	2	5	—
	14 wks	12	3.5-4.5	—		6 wks	12	5-6	—
	16 wks	12	4-4.5	—	White shrimp	10 wks	4	3.5-5.5	—
	18 wks	12	5-6	—	Brown-spotted shrimp	12 wks	12	3-4.5	—
	20 wks	12	5	—	White shrimp	14 wks	12	4.5-5.5	—
Third application (Oct. 27-28, 1970)						16 wks	12	4-5	—
White shrimp	24 hrs	12	5-6	—		18 wks	12	5.5-6	—
	2 wks	3	4.5-6	—		20 wks	10	5-6	—
Brown-spotted shrimp	18 wks	3	3-4.5	—	Third application (Oct. 27-28, 1970)				
	22 wks	5	2-4	—	White shrimp	24 hrs	12	5-6	—
	24 wks	10	3-4	—		2 wks	1	4.5	—
	26 wks	4	3-4.5	—	Brown-spotted shrimp	20 wks	2	5	—
	28 wks	12	3-5.5	—		24 wks	6	3.5-4.5	—
	30 wks	11	2.75-5	—					
	32 wks	12	3-4.5	—					

TABLE 5.—*Mirex* residues in shrimps by sampling site and sampling time, South Carolina, 1969-71—Continued
[— = not detected]

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM— WHOLE BODY)
SAMPLING SITE D—TOOGOODOO CREEK—Continued				
Grass shrimp	24 wks	100	.75-1.25	—
	26 wks	12	2.5-4	—
	28 wks	12	3-4	—
	30 wks	1	4.5	—
	32 wks	6	3.5-5.5	—
STATION 1—RIVERLAND TERRACE POND (EAST)				
Grass shrimp	Background	178	.75-1	—
First application (Dec. 3, 1969)				
Grass shrimp	48 hrs	144	.75-1	—
	5 mos	77	1	—
Grass shrimp	Background	100	.75-1.25	—
Second application (July 24, 1970)				
White shrimp	72 hrs	5	2-3	—
Grass shrimp	3 mos	146	1-1.25	—
Brown-spotted shrimp	3 mos	5	2.25-3	—
Grass shrimp	4 mos.	176	.75-1.25	—
Third application (Dec. 1, 1970)				
Grass shrimp	48 hrs	159	.75-1.25	—
	2 wks	167	.75-1.25	—
	6 wks	193	.75-1.25	—
	3 mos	126	.75-1.25	—
STATION 2—STONO RIVER, LOG BRIDGE CREEK				
White shrimp	Background	13	Medium	—
Brown shrimp	Background	9	Small	—
First application (Oct. 23, 1969)				
White shrimp	24 hrs	4	3-4	—
Second application (June 18, 1970)				
Brown-spotted shrimp	24 hrs	12	4.5-5.5	—
White shrimp	3 mos	12	3.5-4.5	—
STATION 3—UPPER ASHLEY RIVER, RUNNYMEADE PLANTATION				
River shrimp	Background	9	1.5-2.5	—
First application (Oct. 22, 1969)				
Second application (June 11, 1970)				
Brown-spotted shrimp	24 hrs	3	3-4	.11
White shrimp	3 mos	2	4.5	—

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM— WHOLE BODY)
STATION 4—COOPER RIVER, U.S. NAVAL AMMUNITION DEPOT				
First application (Oct. 17, 1969)				
River shrimp	24 hrs	1	3	1.3
White shrimp	24 hrs	5	2.5-3	.26
Second application (June 10, 1970)				
White shrimp	3 mos	10	3-4.5	—
STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK				
White shrimp	Background	6	4	—
First application (Oct. 22, 1969)				
White shrimp	24 hrs	4	3-4	—
Second application (June 10, 1970)				
Brown-spotted shrimp	24 hrs	3	2.5-3	—
White shrimp	3 mos	7	3-6	—
STATION 6—WANDO RIVER, BERESFORD CREEK				
White shrimp	Background	4	4.5-5.5	—
First application (Oct. 17, 1969)				
White shrimp	24 hrs	3	4-6	—
Second application (June 8, 1970)				
Brown-spotted shrimp	24 hrs	12	3.5-4.5	.015
White shrimp	3 mos	12	3.5-4	—
	8 mos	12	3.5-5	—
STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK				
White shrimp	Background	8	2.5-4	—
First application (Sept. 18, 1969)				
White shrimp	24 hrs	9	2-2.5	—
Second application (May 20, 1970)				
Brown-spotted shrimp	3 mos	12	3.5-4	—
White shrimp	8 mos	12	4.5-5	—

TABLE 6.—*Mirex* residues in crabs by sampling site and sampling time, South Carolina, 1969-71
[— = not detected]

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM—WHOLE BODY)	SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM—WHOLE BODY)
SAMPLING SITE A—TOOGOODOO CREEK					SAMPLING SITE B—TOOGOODOO CREEK—Continued				
Blue crab	Background	1	5	—	Third application (Oct. 27-28, 1970)				
First application (Oct. 14-15, 1969)					Blue crab	24 hrs	6	1-2.5	.049
Blue crab	24 hrs	2	3	—	Fiddler crab	24 hrs	30	.33-.75	
	4 wks	5	1-2.5	.12	Blue crab	4 wks	2	3-5	.012
	12 wks	2	4.5-5	.19		6 wks	11	1-2.5	.12
	14 wks	1	4.5	.12	Fiddler crab	6 wks	29	.33-.75	
	16 wks	1	2.5	.19	Blue crab	8 wks	5	1-2	.041
	22 wks	3	1-3	.040		10 wks	4	1.5-5	.027
	26 wks	4	3-4	.015		12 wks	3	.75-3	.038
	28 wks	2	3-4	.026		14 wks	8	.75-2	—
	30 wks	4	3-6	.016		16 wks	9	2-5	.020
Second application (June 3-4, 1970)						18 wks	7	1.25-2	—
Blue crab	24 hrs	3	2-3	—		20 wks	9	1-2	—
	4 wks	5	2.5-5	.19		22 wks	4	2-4	—
	6 wks	3	3-6	—		24 wks	12	1.5-2.5	—
	8 wks	5	2-5	.052		26 wks	4	1-4.5	—
	10 wks	8	5-6	.053		28 wks	5	3-6	—
	12 wks	12	1.5-2.5	—		32 wks	1	3.5	—
	14 wks	12	2-2.5	—	SAMPLING SITE C—TOOGOODOO CREEK				
	16 wks	6	2.5-5.5	—	Blue crab	Background	1	5	—
	18 wks	1	5.5	.024	First application (Oct. 14-15, 1969)				
	20 wks	5	1-5	—	Blue crab	24 hrs	1	6	—
Third application (Oct. 27-28, 1970)						2 wks	1	6	—
Blue crab	24 hrs	3	6	—		4 wks	9	1-2	—
	2 wks	3	1-5	—		6 wks	4	2-3	.015
	4 wks	3	2-5	—		8 wks	1	6	.032
	6 wks	11	1-2	.013		12 wks	2	5.5	.090
	8 wks	12	1-2.5	—		14 wks	1	2	.050
	10 wks	7	1-3	—		18 wks	2	4.5	.056
	14 wks	12	1-1.25	—		20 wks	2	4.5	.025
	16 wks	10	1-1.75	.022		22 wks	8	1-2	.038
	18 wks	6	1.5-2	—		24 wks	2	1-2	—
	20 wks	8	1.25-1.5	—		26 wks	1	3	—
	22 wks	8	1-3	—		28 wks	2	4-5	—
	24 wks	5	3.5-5	.016	Fiddler crab	30 wks	1	4	—
	26 wks	5	1.5-3	—		32 wks	25	.5-1	—
	28 wks	1	2.5	—	Second application (June 3-4, 1970)				
	30 wks	2	3.25-4.5	—	Blue crab	24 hrs	1	4.5	.013
	32 wks	5	2-5.5	—		2 wks	3	6-6.5	—
SAMPLING SITE B—TOOGOODOO CREEK						6 wks	6	3-4.5	.027
Blue crab	Background	1	6	—		8 wks	4	4-5	—
First application (Oct. 14-15, 1969)						12 wks	4	2-4	—
Blue crab	24 hrs	1	6	—		14 wks	1	5	—
	4 wks	1	6	.24		16 wks	2	4.5	—
	8 wks	1	3	.089		20 wks	2	6	—
	14 wks	1	3	.20	Fiddler crab	21 wks	26	.5-.75	—
	18 wks	2	4.5	—	Third application (Oct. 27-28, 1970)				
	26 wks	2	3-4	—	Blue crab	24 hrs	3	3-5	—
	32 wks	3	4-6	.025	Fiddler crab	24 hrs	27	.5-.75	—
Second application (June 3-4, 1970)					Blue crab	2 wks	1	6	—
Blue crab	4 wks	6	2.5-5	.035		4 wks	3	4-5	.010
	8 wks	3	2-6	—		6 wks	4	4.5	—
	10 wks	4	4-5	.017	Fiddler crab	6 wks	31	.5-1	—
	12 wks	12	1.25-2.25	—	Blue crab	8 wks	4	1.5-2.5	—
	14 wks	6	3-5.5	—		14 wks	3	1-1.75	—
	16 wks	1	5	—		16 wks	7	1-2.5	—
	18 wks	1	5.5	—		20 wks	8	1-2	—
Fiddler crab	21 wks	28	.33-.75	—		22 wks	2	1.5-2.5	—
						24 wks	7	1.5-3	—
						26 wks	8	1-2.5	—
						28 wks	8	1.25-3.25	—
						30 wks	1	2	—

TABLE 6.—*Mirex* residues in crabs by sampling site and sampling time, South Carolina, 1969-71—Continued
[— = not detected]

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM—WHOLE BODY)
SAMPLING SITE D—TOOGOODOO CREEK				
Blue crab	Background	1	7	—
First application (Oct. 14-15, 1969)				
Blue crab	24 hrs	6	1-1.5	—
	2 wks	3	1-2	—
	4 wks	12	1-1.25	—
	6 wks	3	1.5-3	.065
	8 wks	12	1-1.75	.030
	12 wks	2	5.5	.051
	14 wks	2	5.5	—
	18 wks	3	1.5-5	—
	20 wks	3	3	—
	22 wks	2	3	—
	24 wks	6	2-3	—
	26 wks	1	4	—
	28 wks	2	4-5	—
	30 wks	1	5	—
	32 wks	2	3-6	—
Second application (June 3-4, 1970)				
Blue crab	24 hrs	5	3-6	—
	4 wks	4	3-4	.098
	6 wks	6	4-6	—
	10 wks	1	4.5	.015
	12 wks	12	1-2.5	—
	14 wks	2	4-5	—
	18 wks	2	5-6	—
	20 wks	3	2-5	—
Third application (Oct. 27-28, 1970)				
Blue crab	24 hrs.	8	1.5-2.5	—
	2 wks	4	2-5	—
	6 wks	5	1.5-4.5	—
	8 wks	12	1-2	—
	10 wks	4	1.5-4.5	—
	12 wks	5	1-5	—
	14 wks	3	1-2	—
	16 wks	12	1-2	—
	20 wks	10	1.5-2.5	—
Mud crab	24 wks	12	.5-1.25	—
Blue crab	26 wks	2	2.5-3.5	—
	28 wks	12	1.75-3.5	—
	30 wks	2	2-3	—
	32 wks	1	2.5	—
STATION 1—RIVERLAND TERRACE POND (EAST)				
Blue crab	Background	3	1-2.5	—
First application (Dec. 3, 1969)				
Blue crab	5 mos	8	.5-1.25	—
Second application (July 24, 1970)				
Blue crab	72 hrs	15	.5-2	.024
	3 mos	13	.75-1.5	—
Third application (Dec. 1, 1970)				
Blue crab	48 hrs	12	.5-2	—
Fiddler crab	48 hrs	4	.5-.75	—
Blue crab	2 wks	3	1-3	.026
	3 mos	12	1.5-4.5	—
STATION 2—STONO RIVER, LOG BRIDGE CREEK				
Blue crab	Background	7	1.5-3	—
First application (Oct. 23, 1969)				
Blue crab	24 hrs	8	2.5-6	.010
	3 mos	2	5-5.5	.031
	8 mos	12	1.25-4	—
STATION 2—STONO RIVER—Continued				
Second application (June 18, 1970)				
Blue crab	24 hrs	8	2.5-6	.010
	3 mos	2	5-5.5	.031
	8 mos	12	1.25-4	—
STATION 3—UPPER ASHLEY RIVER, RUNNYMEADE PLANTATION				
Blue crab	Background	2	1.5	—
First application (Oct. 22, 1969)				
Blue crab	24 hrs	3	2-3	.60
Second application (June 11, 1970)				
Blue crab	24 hrs	4	5	.27
STATION 4—COOPER RIVER, U.S. NAVAL AMMUNITION DEPOT				
Blue crab	Background	1	4	—
First application (Oct. 17, 1969)				
Second application (June 10, 1970)				
Blue crab	24 hrs	4	6-8	.042
	3 mos	1	4.5	—
Mud crab	8 mos	31	.25-.5	—
STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK				
Blue crab	Background	1	5.5	—
First application (Oct. 22, 1969)				
Blue crab	24 hrs	9	1	—
	3 mos	2	1-2.5	—
Second application (June 10, 1970)				
Blue crab	24 hrs	5	1.5-2.5	—
	3 mos	4	3-5	—
	8 mos	8	1-4	—
STATION 6—WANDO RIVER, BERESFORD CREEK				
Blue crab	Background	2	1.5-3	—
First application (Oct. 17, 1969)				
Blue crab	3 mos	2	5	—
Second application (June 8, 1970)				
Blue crab	24 hrs	2	2-5	.025
	3 mos	8	5-6	—
	8 mos	12	1.25-3.25	—
STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK				
Blue crab	Background	1	5.5	—
First application (Sept. 18, 1969)				
Blue crab	24 hrs	1	4.5	—
	3 mos	2	1.5-3	—
Second application (May 20, 1970)				
Blue crab	24 hrs	2	2-3	—
	3 mos	4	4-5	—
	8 mos	12	1-4	.012

TABLE 7.—*Mirex* residues in fishes by sampling site and sampling time, South Carolina, 1969-71
[— = not detected]

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM— WHOLE BODY)
SAMPLING SITE A—TOOGOODOO CREEK				
Blackcheek tonguefish	Background	2	3	—
Southern kingfish	Background	1	7	—
First application (Oct. 14-15, 1969)				
Silver perch	24 hrs	2	3.5	—
	2 wks	2	3.5	—
	4 wks	2	4.5	.073
	8 wks	11	3.5-4.5	.028
Atlantic menhaden	16 wks	10	3.5-4.5	—
	18 wks	10	4-5	—
Spot	30 wks	13	3-4	.015
Second application (June 3-4, 1970)				
Spot	24 hrs	12	3-4	—
	2 wks	7	3.5-5	—
	4 wks	12	4.5-5.5	—
	6 wks	5	3.5-4	—
	8 wks	5	3-4	—
	12 wks	6	2-3	.060
	14 wks	12	4-5	.043
	16 wks	6	3-5	—
Third application (Oct. 27-28, 1970)				
Weakfish	24 hrs	3	4.5-7	—
Silver perch	2 wks	3	4.5-5.5	.017
Striped mullet	6 wks	1	7	—
	10 wks	7	7-9	—
Silver perch	20 wks	2	4.5	—
Atlantic menhaden	20 wks	2	4.25	—
Bay anchovy	22 wks	12	2-3	—
Silver perch	26 wks	4	5.5-7	—
Spot	28 wks	12	1.5-3	—
	30 wks	12	2.25-3.25	—
	32 wks	12	2-3	—
SAMPLING SITE B—TOOGOODOO CREEK				
Silver perch	Background	1	3.5	—
First application (Oct. 14-15, 1969)				
Silver perch	24 hrs	2	3.5	—
	2 wks	1	4.5	—
	8 wks	1	4	—
Spotted seatrout	8 wks	1	7	.039
Atlantic menhaden	14 wks	12	2-4	—
	16 wks	10	3.5-4.5	—
	18 wks	4	4-5	—
Spot	28 wks	3	2-2.5	—
Bluefish	30 wks	1	7	—
Spot	32 wks	2	3-4	—
Second application (June 3-4, 1970)				
Spot	24 hrs	12	3.5-4.5	—
	2 wks	6	3.5-5.5	—
	4 wks	12	3-4.5	—
	6 wks	12	3-4	—
	8 wks	1	3.5	—
Striped mullet	8 wks	2	4-5	—
Silver perch	10 wks	6	4.5-6	.027
Spot	12 wks	12	3.5-4	.016
Silver perch	14 wks	7	4-5	—
Spot	16 wks	4	3-4.5	—
Silver perch	18 wks	4	6-6.5	—
	20 wks	1	5	—
Third application (Oct. 27-28, 1970)				
Silver perch	24 hrs	8	4-5	.034
Spot	2 wks	1	6	—

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM— WHOLE BODY)
SAMPLING SITE B—TOOGOODOO CREEK—Continued				
Striped mullet	6 wks	3	5.5-6.5	.018
Spot	20 wks	4	6.5-7.5	—
Fourspot flounder	22 wks	3	2.5	—
	24 wks	2	3-4	—
Spot	28 wks	12	1.5-2.75	—
	30 wks	5	3-4.5	—
	32 wks	11	2.5-4.25	—
SAMPLING SITE C—TOOGOODOO CREEK				
Blackcheek tonguefish	Background	3	4	—
First application (Oct. 14-15, 1969)				
Hogchoker	24 hrs	1	4	—
Silver perch	2 wks	2	4	—
Blackcheek tonguefish	4 wks	7	4-5	—
Silver perch	6 wks	5	3-4	.017
Atlantic menhaden	14 wks	12	3.5-4.5	—
	20 wks	4	4	—
Mixed fish	22 wks	2	2-4	—
	24 wks	3	2.5-4	—
Searobin	26 wks	1	7	—
Spotted hake	26 wks	1	4	—
Spot	30 wks	3	2-3	—
	32 wks	6	4-6	—
Second application (June 3-4, 1970)				
Spot	24 hrs	10	3.5-4.5	—
	2 wks	2	4-6	—
	4 wks	2	4.5	—
	6 wks	12	3-4	—
Weakfish	8 wks	3	4-5	—
Spot	10 wks	5	3.5-4.5	—
Silver perch	12 wks	12	2.5-3.5	—
	14 wks	12	3-4	—
	16 wks	3	4	—
Spot	18 wks	3	5-6.5	—
Third application (Oct. 27-28, 1970)				
Silver perch	24 hrs	1	4.5	—
	2 wks	2	4	.028
Bay anchovy	26 wks	12	2-3	—
Spot	28 wks	12	2.25-3.5	—
	30 wks	5	3-4.5	—
	32 wks	12	2.5-4.5	—
SAMPLING SITE D—TOOGOODOO CREEK				
Blackcheek tonguefish	Background	2	3	—
First application (Oct. 14-15, 1972)				
Blackcheek tonguefish	24 hrs	2	3.5	—
	2 wks	2	4	—
	4 wks	3	3-4	—
Silver perch	6 wks	10	3.5-4	—
	8 wks	12	3-4	—
Atlantic menhaden	12 wks	2	4	—
	14 wks	10	3.5	—
	16 wks	10	3.5-4.5	—
	18 wks	3	3.5-4	—
Mixed fish	20 wks	2	3-4.5	—
Silver perch	24 wks	1	7	—
Spotted hake	24 wks	2	4	—
Weakfish	26 wks	1	10.5	—
Spotted hake	26 wks	1	8.5	—
Mixed fish	26 wks	3	2-4	—
Spotted hake	28 wks	3	6	—
Bluefish	30 wks	2	3-6	—
Spot	32 wks	7	3-6	—

TABLE 7.—Mirex residues in fishes by sampling site and sampling time, South Carolina, 1969-71—Continued
[— = not detected]

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM— WHOLE BODY)
SAMPLING SITE D—TOOGOODOO CREEK—Continued				
Second application (June 3-4, 1970)				
Spot	24 hrs	12	4.5-5.5	—
	2 wks	8	3-5	—
	4 wks	2	4	—
	10 wks	2	3.5-5.5	—
Silver perch	12 wks	8	2.5-3.5	.046
	14 wks	7	4	—
	16 wks	2	3.5-5	—
Blackcheek tonguefish	18 wks	4	4-5	—
Third application (Oct. 27-28, 1970)				
Silver perch	2 wks	10	4-4.5	—
Winter flounder	8 wks	1	10.5	—
Spotted hake	18 wks	2	3-4.5	—
Spot	20 wks	1	4.5	—
Spotted hake	24 wks	7	3.5-6	—
Silver perch	26 wks	2	3-5	—
Spot	28 wks	12	2-3	—
	30 wks	2	3-3.5	—
	32 wks	12	3-4.5	—

STATION 1—RIVERLAND TERRACE POND (EAST)				
Mummichog	Background	13	1-2	—
White mullet	Background	2	4-5	—
Atlantic silverside	Background	30	1.5-2.5	—
First application (Dec. 3, 1969)				
Atlantic silverside	48 hrs	55	1.5-2	—
Mummichog	12 days	13	1-2.5	—
Atlantic silverside	12 days	49	1.5-2	—
White mullet	5 mos	23	1.25-1.5	—
Second application (July 24, 1970)				
Silver perch	72 hrs	12	2-2.5	—
Sailfin molly	3 mos	17	.5-1	—
Atlantic silverside	4 mos	27	2-3	—
Third application (Dec. 1, 1970)				
Sailfin molly	48 hrs	18	1-1.5	—
White mullet	2 wks	25	.75-1	—
Mummichog	2 wks	20	1.25-2.25	—
Atlantic silverside	2 wks	11	1.75-2	—
	6 wks	25	1.5-2.5	—
White mullet	6 wks	12	2.5-3.75	—
	3 mos	42	1	—

STATION 2—STONO RIVER, LOG BRIDGE CREEK				
Blackcheek tonguefish	Background	4	3	—
Black drum	Background	1	5	—
Spotted seatrout	Background	1	7	—
First application (Oct. 23, 1969)				
Silver perch	24 hrs	2	3-4	—
Second application (June 18, 1970)				
Spot	24 hrs	12	2.5-3.5	—
Silver perch	3 mos	12	4-5	.054
Spot	8 mos	21	1.5-2.5	—

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM— WHOLE BODY)
STATION 3—UPPER ASHLEY RIVER, RUNNYMEADE PLANTATION				
Atlantic croaker	Background	1	5	—
Hog choker	Background	3	3	—
First application (Oct. 22, 1969)				
Hog choker	24 hrs	4	1-2.5	—
	2 mos	8	2-3	.053
White catfish	2 mos	6	2-3	.35
	3 mos	1	12.5	.086
Striped mullet	3 mos	1	8	—
Second application (June 11, 1970)				
Spot	24 hrs	8	3-5	.82
Silver perch	3 mos	3	4	.20
Hog choker	3 mos	8	2-2.5	.096
White catfish	8 mos	7	3.5-7	.19

STATION 4—COOPER RIVER, U.S. NAVAL AMMUNITION DEPOT				
Spot	Background	3	2	—
First application (Oct. 17, 1969)				
Silver perch	24 hrs	2	3.5	.21
Mixed fish	2 mos	2	4-6	—
White catfish	3 mos	1	13	.036
Second application (June 10, 1970)				
Spot	24 hrs	9	2-3.5	.12
Hogchoker	24 hrs	6	1.5-2	—
Silver perch	3 mos	5	5-5.5	.14
White catfish	8 mos	1	5.5	.045
Spot	8 mos	2	4.5-5.5	—
Bay anchovy	8 mos	14	1.5-2.25	—

STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK				
Spot	Background	3	3.5	—
Winter flounder	Background	1	8	—
First application (Oct. 22, 1969)				
Blackcheek tonguefish	24 hrs	3	3	—
Atlantic menhaden	3 mos	12	2-4	—
Second application (June 10, 1970)				
Spot	24 hrs	8	2-3	—
Silver perch	3 mos	9	4-4.5	—
Atlantic menhaden	8 mos	5	4.5	—
Striped mullet	8 mos	2	5.5	—
Star drum	8 mos	43	1-1.75	—

TABLE 7.—*Mirex residues in fishes by sampling site and sampling time, South Carolina, 1969-71*—Continued
[— = not detected]

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM—WHOLE BODY)	SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM—WHOLE BODY)
STATION 6—WANDO RIVER, BERESFORD CREEK					STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK				
Silver perch	Background	2	4.5	—	Atlantic croaker	Background	2	4	—
First application (Oct. 17, 1969)					Hog choker	Background	4	2-2.5	—
Pinfish	24 hrs	1	7	—	Spot	Background	3	2-3	—
Second application (June 8, 1970)					First application (Sept. 18, 1969)				
Spot	24 hrs	12	2.5-3.5	.016	Spot	24 hrs	3	2-3	—
	3 mos	12	3.5-5	—	Sheepshead	3 mos	1	8.5	—
Silver perch	8 mos	22	1.5-2	—	Second application (May 20, 1970)				
					Spot	24 hrs	2	6	—
					Silver perch	24 hrs	1	5	—
						3 mos	6	3.5-6.5	—
					Spot	8 mos	12	3.75-4.5	.011

TABLE 8.—*Mirex residues in miscellaneous organisms by sampling site and sampling time, South Carolina, 1969-71*
[— = not detected]

SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM) ¹	SPECIES	INTERVAL FROM MIREX APPLICATION TO SAMPLING	NUMBER IN COMPOSITE SAMPLE	SIZE OF INDIVIDUALS IN COMPOSITE SAMPLES (INCHES)	MIREX RESIDUE (PPM) ¹
SAMPLING SITE A—TOOGOODOO CREEK					SAMPLING SITE D—TOOGOODOO CREEK				
Second application (June 3-4, 1970)					Second application (June 3-4, 1970)				
American oyster	12 wks	12	2-4	—	American oyster	12 wks	12	2-4	—
SAMPLING SITE B—TOOGOODOO CREEK					STATION 1—RIVERLAND TERRACE POND (EAST)				
First application (Oct. 14-15, 1969)					First application (Dec. 3, 1969)				
Brief squid	32 wks	10	4-6	—	Egg masses from 186 gravid grass shrimp	5 mos			—
Second application (June 3-4, 1970)					Third application (Dec. 1, 1970)				
American oyster	12 wks	12	2-4	—	Dead white mullet	2 wks	1	5	—
Third application (Oct. 27-28, 1970)									
American oyster	6 wks	28	2-4	—					
	10 wks	18	2-4	—					
	12 wks	10	2-4	—					
SAMPLING SITE C—TOOGOODOO CREEK					STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK				
First application (Oct. 14-15, 1969)					Second application (June 10, 1970)				
American oyster	32 wks	25	2-4	—	Dead striped mullet	24 hrs	1	11.5	—
Southern periwinkle	32 wks	40	.5-.75	—					
Phytoplankton (dry weight)	32 wks			—					
Second application (June 3-4, 1970)					STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK				
American oyster	12 wks	12	2-4	—	First application (Oct. 18, 1969)				
Third application (Oct. 27-28, 1970)					Pied-billed grebe (breast muscle) (drowned in net)	3 mos			—
American oyster	6 wks	22	2-4	—					
	10 wks	13	2-4	—					
	12 wks	12	2-4	—					
Nudibranch	16 wks	18	.5-1	—					

¹ Residues are whole-body basis unless otherwise indicated.

TABLE 9.—*Mirex* residues in birds by sampling site and sampling time, South Carolina, 1969-71
[— = not detected]

QUARTERLY COLLECTION	SPECIES	MIREX RESIDUE (PPM) ¹	QUARTERLY COLLECTION	SPECIES	MIREX RESIDUE (PPM) ¹
SAMPLING SITES A-B-C—TOOGOODOO CREEK			SAMPLING SITE D—TOOGOODOO CREEK—Continued		
December 1969	Green Heron	.99	May 1970	Plover	—
	Kingfisher	1.30		Clapper Rail	—
	Clapper Rail	.29		do.	.61
	do.	1.40	September 1970	Kingfisher	—
	do.	.05		do.	1.50
	do.	.10		do.	.04
	do.	.15		Clapper Rail	—
	do.	1.90		do.	—
	do.	.29		do.	—
	Oil glands from 5 Clapper Rails	.52	December 1970	Clapper Rail	.06
March 1970	Clapper Rail	—		do.	.03
	do.	.06		do.	.03
	do.	.05	February 1971	Clapper Rail	.06
	do.	.08		Kingfisher	—
	do.	.10	May 1971	Snowy Egret	.17
	do.	.06		Green Heron	.04
	Oil glands from 6 Clapper Rails	.29		Louisiana Heron	—
May 1970	Green Heron	.09		do.	.03
	do.	.17	STATION 2—STONO RIVER, LOG BRIDGE CREEK		
	do.	—	September 1969	American Egret	—
	do.	.07		Little Blue Heron	.12
	Yellow Crowned Night Heron	.11		Clapper Rail	.19
	Little Blue Heron	—	December 1969	Clapper Rail	.55
	Clapper Rail	.11		do.	.90
September 1970	Clapper Rail	—	March 1970	Clapper Rail	—
	do.	.17		do.	.18
	do.	—		do.	.11
	do.	.02		Sora Rail	—
	do.	—	May 1970	Clapper Rail	.08
	do.	.02		do.	.05
	do.	.05	September 1970	Clapper Rail	.10
	do.	.63		do.	.21
	do.	.02		do.	.16
	do.	.19	December 1970	Clapper Rail	.11
	do.	.04		do.	.06
	do.	—	February 1971	Clapper Rail	.09
December 1970	Clapper Rail	.15		do.	.13
	do.	—	May 1971	Clapper Rail	.09
	10 Rails (Muscle)	.07		Little Blue Heron	.70
	10 Rails (Fat)	1.20	STATION 3—UPPER ASHLEY RIVER, RUNNYMEADE PLANTATION		
	Willet	—	September 1969	Green Heron	.25
	Kingfisher	.15		do.	.15
February 1971	Clapper Rail	.04		do.	.13
	do.	.13	December 1969	Snowy Egret	.69
	do.	.10		American Bittern	1.80
	do.	.03	March 1970	Snipe	.11
	do.	.19		do.	1.10
	do.	.04	May 1970	Anhinga (Juvenile)	—
	do.	.03		Anhinga	1.70
	do.	.07		Kingfisher	17.00
	do.	.05		(internal organs)	8.30
	do.	.75	September 1970	Pied-Billed Grebe	.28
	do.	—		Anhinga	.35
	Oil glands from 6 Clapper Rails	.58	December 1970	Snipe	.05
	Louisiana Heron	.11		do.	.34
May 1971	Clapper Rail	.01		do.	.25
	Louisiana Heron	.94		do.	.59
	do.	.14	SAMPLING SITE D—TOOGOODOO CREEK		
	Little Blue Heron	.01	December 1969	Attempts to collect birds unsuccessful	—
	do.	.22	March 1970	Clapper Rail	—
	American Egret	5.40		do.	.11
	do.	.05			

TABLE 9.—*Mirex* residues in birds by sampling site and sampling time, South Carolina, 1969-71—Continued
[— = not detected]

QUARTERLY COLLECTION	SPECIES	MIREX RESIDUE (PPM) ¹
STATION 3—UPPER ASHLEY RIVER—Continued		
February 1971	Snowy Egret	.60
	Marsh Hawk	1.50
May 1971	Least Bittern	2.20
	Louisiana Heron	1.10
STATION 4—COOPER RIVER, U.S. NAVAL AMMUNITION DEPOT		
September 1969	Louisiana Heron	—
	do.	—
December 1969	Attempts to collect birds unsuccessful	
March 1970	Snipe	.14
	do.	.89
May 1970	Grebe	—
September 1970	Louisiana Heron	.38
	Green Heron	1.00
December 1970	Clapper Rail	.60
	do.	.94
February 1971	Clapper Rail	.19
	American Bittern	.04
	Grebe	1.10
May 1971	Least Bittern	2.80
	Clapper Rail	.18

STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK		
September 1969	Louisiana Heron	—
	American Egret	—
December 1969	Clapper Rail	.11
	do.	.35
March 1970	Clapper Rail	.09
	do.	.07
	Sora Rail	.09
	Virginia Rail	.06
May 1970	Clapper Rail	—
	do.	—
	Eggs	.08
September 1970	Clapper Rail	—
	do.	.04
December 1970	Clapper Rail	.07
	do.	.06
February 1971	Clapper Rail	.09
	Sora Rail	.01

COLLECTION QUARTERLY	SPECIES	MIREX RESIDUE MIREX
STATION 5—LOWER ASHLEY RIVER—Continued		
May 1971	Clapper Rail	.10
	do.	—
	do.	.04
	Eggs	.05
STATION 6—WANDO RIVER, BERESFORD CREEK		
September 1969	Snowy Egret	.89
	American Egret	—
December 1969	Attempts to collect birds unsuccessful....	
March 1970	Clapper Rail	.05
	Snipe	—
May 1970	Clapper Rail	.47
	White Ibis	.29
September 1970	Kingfisher	—
	do.	—
	Sora Rail	—
	Clapper Rail	.04
	do.	—
December 1970	Clapper Rail	.82
	do.	.25
February 1971	Clapper Rail	.71
	do.	.07
May 1971	Green Heron	.14
	Little Blue Heron	.17

STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK		
September 1969	Snowy Egret	—
	Little Blue Heron	—
December 1969	Clapper Rail	—
	do.	—
March 1970	Grebe	—
May 1970	Snowy Egret	—
	do.	.20
September 1970	Kingfisher	—
	do.	.06
December 1970	Snowy Egret	.51
	American Egret	.45
February 1971	Snowy Egret	.13
	Louisiana Heron	.17
May 1971	Clapper Rail	.10
	Snowy Egret	.11

¹ Residues are breast and upper wing muscle unless otherwise indicated.

TABLE 10.—Average mirex residues in muscle tissue of herons and egrets at each station, South Carolina, 1969-71

STATION	AVERAGE RESIDUES IN PPM AND NUMBER OF BIRDS ()							
	Sept. 1969	Dec. 1969	Mar. 1970	May 1970	Sept. 1970	Dec. 1970	Feb. 1971	May 1971
A-B-C		.99 (1)		.07 (6)			.11 (1)	1.13 (6)
D								.06 (4)
2	.06 (2)							.70 (1)
3	.18 (3)	.69 (1)					.60 (1)	1.10 (1)
4	.00 (2)				.69 (2)			
5	.00 (2)							
6	.45 (2)							.16 (2)
7	.00 (2)			.10 (2)		.48 (2)	.15 (2)	.11 (1)
Overall average	.12	.84		.08	.69	.48	.25	.61
Total birds	(13)	(2)		(8)	(2)	(2)	(4)	(15)

TABLE 11.—Average mirex residues in muscle tissue of clapper rails at each station, South Carolina, 1969-71

STATION	AVERAGE RESIDUES IN PPM AND NUMBER OF BIRDS ()							
	Sept. 1969	Dec. 1969	Mar. 1970	May 1970	Sept. 1970	Dec. 1970	Feb. 1971	May 1971
A-B-C		.60 (7)	.07 (6)	.11 (1)	.09 (13)	.07 (12)	.14 (11)	.01 (1)
D			.06 (2)	.31 (2)	.00 (3)	.04 (3)	.06 (1)	
2	.19 (1)	.73 (2)	.10 (3)	.07 (2)	.16 (3)	.08 (2)	.11 (2)	.09 (1)
3								
4						.77 (2)	.19 (1)	.18 (1)
5		.23 (2)	.08 (2)	.00 (2)	.02 (2)	.06 (2)	.09 (1)	.05 (3)
6			.05 (1)	.47 (1)	.02 (2)	.54 (2)	.39 (2)	
7	.00 (2)							.10 (1)
Overall average	.19	.47	.07	.16	.07	.23	.15	.07
Total birds	(1)	(13)	(14)	(8)	(23)	(23)	(18)	(7)

TABLE 12.—Mirex residues in mammals by sampling site and sampling time, South Carolina, 1969-71
[— = not detected]

QUARTERLY COLLECTION	SPECIES	MIREX RESIDUE (PPM) ¹
SAMPLING SITE A—TOOGOODOO CREEK		
December 1969	Raccoon	.14
	Fat	.97
March 1970	Raccoon	.05
May 1970	Raccoon	1.30
	Raccoon	.20
September 1970	Raccoon	.02
	Raccoon	.26
December 1970	Raccoon	.02
	Raccoon	—
February 1971	Raccoon	—
	Raccoon	.16
May 1971	Raccoon	.88
	Raccoon	—
SAMPLING SITE B—TOOGOODOO CREEK		
December 1969	Raccoon	—
	Fat	—
	Raccoon	—
	Fat	.16
March 1970	Raccoon	.07
	Raccoon	.43
May 1970	Raccoon	.03
	Raccoon	.66
	Raccoon	—
	Raccoon	.12

QUARTERLY COLLECTION	SPECIES	MIREX RESIDUE (PPM) ¹
SAMPLING SITE B—TOOGOODOO CREEK—Continued		
September 1970	Raccoon	.21
	Raccoon	—
December 1970	Raccoon	—
	Raccoon	—
February 1971	Raccoon	.04
	Raccoon	.12
May 1971	Raccoon	.16
	Raccoon	.04
SAMPLING SITE C—TOOGOODOO CREEK		
December 1960	Raccoon	—
	Raccoon	—
March 1970	Attempts to collect raccoons unsuccessful.	
May 1970	Attempts to collect raccoons unsuccessful.	
September 1970	Raccoon	.15
	Raccoon	.24
December 1970	Raccoon	.02
	Raccoon	.08
February 1971	Raccoon	.04
	Raccoon	.09
May 1971	Raccoon	.09
	Raccoon	.02

TABLE 12.—*Mirex* residues in mammals by sampling site and sampling time, South Carolina, 1969-71—Continued
[— = not detected]

QUARTERLY COLLECTION	SPECIES	MIREX RESIDUE (PPM) ¹	QUARTERLY COLLECTION	SPECIES	MIREX RESIDUE (PPM) ¹
SAMPLING SITE D—TOOGOODOO CREEK			STATION 4—COOPER RIVER—Continued		
December 1969	Raccoon	—	September 1970	Raccoon	.90
	Raccoon	—		Raccoon	1.30
	Fat	—	December 1970	..Attempts to collect raccoons unsuccessful..	
March 1970	..Attempts to collect raccoons unsuccessful..		February 1971	..Attempts to collect raccoons unsuccessful..	
May 1970	Raccoon	.01	May 1971	..Attempts to collect raccoons unsuccessful..	
	Raccoon	.04	STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK		
September 1970	Raccoon	.01	December 1969	Raccoon	—
	Raccoon	—		Raccoon	—
December 1970	Raccoon	—	March 1970	Raccoon	—
	Raccoon	—		Raccoon	—
February 1971	Raccoon	—	May 1970	Raccoon	—
	Raccoon	.04		Raccoon	—
May 1971	Raccoon	—	September 1970	Raccoon	.06
	Raccoon	—		Raccoon	—
STATION 2—STONO RIVER, LOG BRIDGE CREEK			December 1970	Raccoon	.03
December 1969	Raccoon	—		Raccoon	.02
	Fat	—	February 1971	Raccoon	—
	Raccoon	—		Raccoon	—
	Fat	—	May 1971	Raccoon	—
March 1970	Raccoon	—		Raccoon	.05
May 1970	Raccoon	1.40	STATION 6—WANDO RIVER, BERESFORD CREEK		
	Raccoon	.07	December 1969	Raccoon	.04
September 1970	Raccoon	.07		Raccoon	—
	Raccoon	1.90	March 1970	Raccoon	—
December 1970	Raccoon	—		Raccoon	—
	Raccoon	—	May 1970	Raccoon	—
February 1971	Raccoon	.20		Raccoon	.16
	Raccoon	.04	September 1970	Raccoon	—
May 1971	Raccoon	—	December 1970	Raccoon	.02
STATION 3—UPPER ASHLEY RIVER, RUNNYMEADE PLANTATION				Raccoon	.02
December 1969	..Attempts to collect raccoons unsuccessful..		February 1971	Raccoon	.04
March 1970	..Attempts to collect raccoons unsuccessful..			Raccoon	—
May 1970	Raccoon	—	May 1971	Raccoon	—
September 1970	Raccoon	.56		Opossum	.80
	Raccoon	.12		Opossum	2.20
	Raccoon	.88	STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK		
December 1970	Raccoon	.10	September 1969	Raccoon	—
	Raccoon	—	December 1969	Raccoon	—
February 1971	Raccoon	.19		Raccoon	—
May 1971	Raccoon	1.90	March 1970	Raccoon	—
	Raccoon	.09		Raccoon	—
	Opossum	3.30	May 1970	..Attempts to collect raccoons unsuccessful..	
STATION 4—COOPER RIVER, U.S. NAVAL AMMUNITION DEPOT			September 1970	..Attempts to collect raccoons unsuccessful..	
December 1969	Raccoon	.80	December 1970	Raccoon	—
	Raccoon	.39		Raccoon	—
March 1970	Raccoon	.60	February 1971	Raccoon	.06
	Raccoon	4.40		Raccoon	—
May 1970	Raccoon	.28	May 1971	Raccoon	.03

¹ Residues for thigh muscles unless otherwise indicated.

TABLE 13.—Average mirex residues in muscle tissue of raccoons at each station, South Carolina, 1969-71

STATION	AVERAGE RESIDUES IN PPM AND NUMBER OF RACCOONS ()							
	Sept. 1969	Dec. 1969	Mar. 1970	May 1970	Sept. 1970	Dec. 1970	Feb. 1971	May 1971
A		.14 (1)	.05 (1)	.75 (2)	.14 (2)	.01 (2)	.08 (2)	.44 (2)
B		.00 (2)	.25 (2)	.20 (4)	.11 (2)	.00 (2)	.08 (2)	.10 (2)
C		.00 (2)			.20 (2)	.05 (2)	.07 (2)	.06 (2)
D		.00 (2)		.03 (2)	.01 (2)	.00 (2)	.02 (2)	.00 (2)
2		.00 (2)	.00 (1)	.74 (2)	.99 (2)	.00 (2)	.12 (2)	.00 (2)
3				.00 (1)	.52 (3)	.05 (2)	.19 (1)	1.00 (2)
4		.60 (2)	2.50 (2)	.28 (1)	1.10 (2)			
5		.00 (2)	.00 (2)	.00 (2)	.03 (2)	.03 (2)	.00 (2)	.03 (2)
6		.02 (2)	.00 (2)	.08 (2)	.00 (1)	.02 (2)	.02 (2)	.20 (1)
7	.00 (1)	.00 (2)	.00 (2)			.00 (2)	.03 (2)	.03 (1)
Overall average	.00	.08	.46	.27	.37	.02	.06	.22
Total raccoons	(1)	(17)	(12)	(16)	(18)	(18)	(17)	(15)

TABLE 14.—Summary of birds and mammals containing mirex residues in excess of 1.0 ppm, South Carolina, 1969-71

STATION	ANIMAL SPECIES	MIREX RESIDUES IN PPM	MONTH OF SAMPLE COLLECTION
BIRDS			
A-B-C	Kingfisher	1.30	December 1969
A-B-C	Clapper Rail	1.40	December 1969
A-B-C	Clapper Rail	1.90	December 1969
A-B-C	American Egret	5.40	May 1971
D	Kingfisher	1.50	September 1970
3	American Bittern	1.80	December 1969
3	Snipe	1.10	March 1970
3	Anhinga	1.70	May 1970
3	Kingfisher	17.00	May 1970
3	Marsh Hawk	1.50	February 1971
3	Least Bittern	2.20	May 1971
3	Louisiana Heron	1.10	May 1971
4	Louisiana Heron	1.00	September 1970
4	Grebe	1.10	February 1971
4	Least Bittern	2.80	May 1971
MAMMALS			
A	Raccoon	1.30	May 1970
2	Raccoon	1.40	May 1970
2	Raccoon	1.90	September 1970
3	Raccoon	1.90	May 1971
3	Opossum	3.30	May 1971
4	Raccoon	4.40	March 1970
4	Raccoon	1.30	September 1970
6	Opossum	2.20	May 1971

Eggshell Thinning, Chlorinated Hydrocarbons, and Mercury in Inland Aquatic Bird Eggs, 1969 and 1970¹

Raymond A. Faber and Joseph J. Hickey

ABSTRACT

In the Upper Great Lakes States, 9 out of 13 species of fish-eating birds were found in 1969-70 to have sustained statistically significant decreases in eggshell thickness since 1946. Maximum changes in a thickness index occurred in great blue herons (-25%), red-breasted mergansers (-23%), common mergansers (-15%), and double-crested cormorants (-15%). Heron eggs taken in Louisiana generally displayed a smaller post-'46 change than herons in the Middle West. On a lipid basis, mean PCB- and DDE-residue levels exceeded 100 ppm in 7 out of 13 species in the Great Lakes States, and in 2 of 7 species in Louisiana, the average DDE:PCB ratios in the two regions being 1.25:1 and 3.9:1, respectively. Individual dieldrin values tended to be higher in Louisiana (31.6 and 13.95 ppm in heron species from two different locations), although values reached 10.1 and 9.4 ppm in great blue and black-crowned night herons in Wisconsin. BHC averaged 3.01 and 0.39 ppm in the Lake States and Louisiana, respectively. Of eggs examined for mercury, 29% had levels greater than 0.5 ppm, and 9%, greater than 1.0 ppm on a wet-weight basis. Mercury levels in a small sample of eggs from Louisiana were consistently low. The differences in mercury levels between the two regions thus were similar to those found for the chlorinated hydrocarbons. While DDE was a prominent factor for most groups, especially herons, in relation to the eggshell thinning observed, dieldrin was also important to two groups even though DDE was present in much higher amounts. PCB's were also important to mergansers, while mercury was positively correlated with thickness index in grebes and negatively correlated in mergansers.

Introduction

This report summarizes the levels of chlorinated hydrocarbons and mercury in 117 eggs of 19 species of aquatic-feeding birds collected in 1969 and 1970 in the Upper Great Lakes States (Wisconsin, Michigan, and Minnesota) and in Louisiana. Shell thickness and a thickness index (24) for these 117 eggs are compared to museum data for eggs collected before 1947. Of the 30 species of raptorial and fish-eating birds in which the eggshell-thinning phenomenon has now been found in great Britain (25) and North America (1), 6 species are known to have DDE associated with this phenomenon (2, 4, 7, 9, 13, 16, 32). Blus *et al.* (4) used multiple regression to obtain a predictive equation for shell thickness of brown pelican eggs (*Pelecanus occidentalis*) in which DDE was the most important factor. Laboratory confirmation of this DDE effect has been provided by Heath *et al.* (14), Bitman *et al.* (3), and Wiemeyer and Porter (33). The effect of dieldrin on eggshell thinning has also been reported by Lehner and Egbert (22) and Enderson and Berger (9).

Because of the combined presence of the chlorinated hydrocarbons in animal tissues, partial-correlation analysis (28, 29) was used in this study to determine the importance of each chemical, including mercury, to eggshell-thickness changes in the 6 families and 19 species in our samples. Chemical residues in birds' eggs, of course, only reflect levels in the females that laid the eggs and are not necessarily the cause of shell-thinning.

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Methods

FIELD COLLECTIONS

All eggs were collected in 1970 except for those of the redbreasted merganser which were collected in 1969. Sites for collection within the Great Lakes area and Louisiana were selected largely on the basis of availability rather than with regard to probably "polluted" areas, except in the case of the herring gull. Herring gull eggs came from upper Green Bay, Wis., where eggs in previous years have had high levels of DDE (20). Only one viable egg was collected from each active nest, but all eggs were collected from nests containing only addled or abandoned eggs. All eggs were wrapped in aluminum foil and frozen until analysis.

EGGSHELL MEASUREMENTS

Eggshell measurements of pre-1947 eggs were taken from specimens in the collections of five museums and one private collector. All eggshells were measured fol-

lowing the methods of Hickey and Anderson (16). In order to increase sample size, we first compared pre-1947 data from different regions; if no significant differences were found, we combined the samples to form a larger one for comparison with the 1970 data. These data are presented in Table 1. For some species, a few eggs collected after 1946 (usually 1958 or after) were found in these collections. In these cases, comparisons were made in two ways, i.e., pre-1947 with 1970 and pre-1947 with 1970 and the post-1946 museum eggs combined.

In addition to shell thickness, we relied on a statistic—thickness index—initially used by Ratcliffe (24) and calculated as shell weight divided by the product of egg length times breadth. The thickness index was used to measure eggshell changes for two reasons: (1) it seems to be less subject to variation in the measuring technique when many species with very different eggshell char-

TABLE 1.—Comparison of pre-1947 eggshell thickness and thickness index with 1970 data and with 1970 and post-1946 museum egg data combined

COMMON AND SCIENTIFIC NAME	COLLECTION REGION	MEAN THICKNESS IN MM $\pm$ 95% CONFIDENCE LEVEL			MEAN THICKNESS INDEX $\pm$ 95% CONFIDENCE LEVEL		
		(N) PRE-1947	(N) 1970 ^{1/} / (N) 1970 AND Post-1946 Museum Egg Data Combined	PERCENT CHANGE	(N) PRE-1947	(N) 1970 ^{1/} / (N) 1970 AND Post-1946 Museum Egg Data Combined	PERCENT CHANGE
Red-necked grebe (<i>Podiceps griseogen</i>)	Wisconsin	(112) 0.357 $\pm$.005	(2) 0.350 $\pm$.00	— 2.0	(112) 1.84 $\pm$.02	(3) 1.62 $\pm$.21	— 13.0*
	Wisconsin and Ontario		(6) 0.338 $\pm$.015	— 5.3		(7) 1.68 $\pm$.10	— 8.7**
Pied-billed grebe (<i>Podilymbus podiceps</i>)	Wisconsin	(71) 0.286 $\pm$.005	(9) 0.288 $\pm$.021	+ 0.7	(71) 1.47 $\pm$.02	(9) 1.45 $\pm$.12	— 1.4
	Wisconsin					(17) 1.42 $\pm$.06	— 3.4
White pelican (<i>Pelecanus erythrorhynchos</i>)	Minnesota	² (92) 0.683 $\pm$.010	(10) 0.633 $\pm$.040	— 7.3*	² (94) 3.33 $\pm$.05	(10) 3.12 $\pm$.22	— 6.3
Double-crested cormorant (<i>Phalacrocorax auritus</i>)	Minnesota	² (350) 0.430 $\pm$.003	(19) 0.370 $\pm$.014	— 14.0*	² (370) 2.12 $\pm$.02	(19) 1.80 $\pm$.08	— 15.1*
Great blue heron (<i>Ardea herodias</i>)	Wisconsin	² (170) 0.393 $\pm$.004	(5) 0.330 $\pm$.029	— 16.0*	² (288) 2.05 $\pm$.02	(5) 1.54 $\pm$.18	— 24.9**
Common egret (<i>Casmerodius albus</i>)	Wisconsin	(235) 0.295 $\pm$.003	(4) 0.275 $\pm$.062	— 6.8	(235) 1.49 $\pm$.01	(4) 1.38 $\pm$.34	— 7.4*
Black-crowned night heron (<i>Nycticorax nycticorax</i>)	Wisconsin	² (134) 0.287 $\pm$.003	(6) 0.272 $\pm$.023	— 5.2	² (158) 1.44 $\pm$.02	(6) 1.30 $\pm$.14	— 9.7
American bittern (<i>Botaurus lentiginosus</i>)	Wisconsin	(68) 0.243 $\pm$.004	(1) 0.24	0	(72) 1.22 $\pm$.02	(1) 1.18	— 3.3
Hooded merganser (<i>Lophodytes cucullatus</i>)	Wisconsin	(44) 0.614 $\pm$.019	(11) 0.599 $\pm$.035	— 2.4	(44) 4.00 $\pm$.11	(11) 3.82 $\pm$.24	— 4.5
Common merganser (<i>Mergus merganser</i>)	Wisconsin and Michigan	(45) 0.437 $\pm$.014	(13) 0.368 $\pm$.012	— 15.8**	(62) 2.49 $\pm$.06	(13) 2.11 $\pm$.08	— 15.3*

acteristics are considered; and (2) there seems to be a change in density of the shell in very thin-shelled eggs in some species (10).

CHEMICAL ANALYSIS

All eggs were analyzed for chlorinated hydrocarbons by WARF Institute, Inc., Madison, Wis., using gas chromatography. Methods of analysis followed those given by Anderson *et al.* (2) with the exception described here. A Barber-Coleman Pesticide Analyzer Model 5360 with two columns was used: one column was packed with 5% DC-200 on 60/70-mesh Chromport XXX, with a column temperature of 200°C and a nitrogen flow rate such that *p,p'*-DDT had a retention time of 6-8 minutes. The other column was packed with 3% OV-17 on 100/120-mesh Gas Chrom Q, with a column temperature of 195°C, and a flow rate such that lindane had a retention time of 1 minute. The estimated polychlori-

nated biphenyl (PCB) values were determined from the heights of the peak between TDE and DDT and from the peak at DDT after saponification. Recovery rates were 80-90%.

The 86 eggs analyzed for mercury included all eggs from the Great Lakes area (one herring gull sample was lost) and 1 egg per species, chosen randomly, from the 7 species of herons from Louisiana. Five black tern eggs were analyzed by the Gulf Radiation Technology Division, Gulf Energy and Environmental Systems, Inc., using neutron-activation analysis, the samples analyzed being mostly albumen mixed with a small amount of yolk. Weighed portions of each sample were sealed in vials and irradiated at a flux of 10^{12} thermal neutrons per cm^2 per second together with a mercury comparator standard. The irradiated samples were digested, in the presence of mercury carrier, in a mixture of HNO_3 and

TABLE 1.—Comparison of pre-1947 eggshell thickness and thickness index with 1970 data and with 1970 and post-1946 museum egg data combined—Continued

COMMON AND SCIENTIFIC NAME	COLLECTION REGION	MEAN THICKNESS IN MM $\pm$ 95% CONFIDENCE LEVEL			MEAN THICKNESS INDEX $\pm$ 95% CONFIDENCE LEVEL		
		(N) PRE-1947	(N) 1970 ¹ / (N) 1970 AND Post-1946 Museum Egg Data Combined	PERCENT CHANGE	(N) PRE-1947	(N) 1970 ¹ / (N) 1970 AND Post-1946 Museum Egg Data Combined	PERCENT CHANGE
Red-breasted merganser (<i>Mergus serrator</i>)	Wisconsin	(141) 0.365 $\pm$.003	(11) 0.303 $\pm$.013	— 17.0**	(148) 2.01 $\pm$.02	(11) 1.55 $\pm$.08	— 22.9**
Herring gull (<i>Larus argentatus</i>)	Wisconsin	^a (369) 0.375 $\pm$.002	(10) 0.328 $\pm$.021	— 12.5*	^a (456) 1.73 $\pm$ 0.01	(10) 1.49 $\pm$.10	— 13.9*
Black tern (<i>Chlidonias nigra</i>)	Wisconsin	(91) 0.155 $\pm$.002	(5) 0.132 $\pm$.022	— 14.8**	(91) 0.69 $\pm$.01	(5) 0.62 $\pm$.08	— 10.1**
Green heron (<i>Butorides virescens</i>)	Louisiana	(100) 0.176 $\pm$.002	(3) 0.163 $\pm$.038	— 7.4	(105) 0.89 $\pm$.01	(3) 0.81 $\pm$.31	— 9.0*
	Louisiana and South Carolina		(11) 0.166 $\pm$.008	— 5.7*		(11) 0.83 $\pm$.05	— 6.7**
Little blue heron (<i>Florida caerulea</i>)	Louisiana	(18) 0.239 $\pm$.007	(5) 0.222 $\pm$.030	— 7.1	(18) 1.14 $\pm$.04	(5) 1.09 $\pm$.10	— 4.4
Cattle egret (<i>Bubulcus ibis</i>)	Louisiana	(7) 0.236 $\pm$.007	(5) 0.228 $\pm$.010	— 3.4	(7) 1.10 $\pm$.05	(5) 1.06 $\pm$.06	— 3.6
	Louisiana and South Carolina		(20) 0.228 $\pm$.007	— 3.4		(20) 1.07 $\pm$ 0.3	— 2.7
Common egret (<i>Casmerodius albus</i>)	Louisiana	(235) 0.295 $\pm$.003	(5) 0.292 $\pm$.013	— 1.0	(235) 1.49 $\pm$.01	(5) 1.44 $\pm$.08	— 3.4
Snowy egret (<i>Leucophoyx thula</i>)	Louisiana	(85) 0.235 $\pm$.004	(5) 0.222 $\pm$ 0.27	— 5.5	(85) 1.14 $\pm$.02	(5) 1.08 $\pm$.11	— 5.3
	Louisiana and South Carolina		(9) 0.220 $\pm$.014	— 6.4*		(9) 1.07 $\pm$.06	— 6.1*
Louisiana heron (<i>Hydranassa tricolor</i>)	Louisiana	(8) 0.240 $\pm$.013	(5) 0.236 $\pm$.030	— 1.7	(8) 1.19 $\pm$.05	(5) 1.14 $\pm$.14	— 4.2
Yellow-crowned night heron (<i>Nyctanassa violacea</i>)	Louisiana	(71) 0.287 $\pm$.004	(5) 0.282 $\pm$.027	— 1.7	(71) 1.45 $\pm$.02	(5) 1.44 $\pm$.16	— 0.7

¹ Red-breasted merganser collected in 1969.

² Pre-1974 data from Anderson and Hickey (1).

* Significant difference ($P < 0.00$.) by Student's t-test.

** Highly significantly different ($P < 0.005$)

H₂SO₄ under reflux conditions. Radiochemical procedures were used to isolate mercury as the metal. Multichannel gamma-ray spectrometry was used to identify and quantitate mercury. The remaining eggs were analyzed for mercury by WARF, Inc., using the method in *The Analyst* (19), modified by atomic absorption spectrophotometry with cold vapor.

These two methods measure total mercury, both organic and inorganic. Because of the biological methylation of mercury (18, 21), and the biological route that mercury takes in reaching eggs, we feel it is safe to assume that most of the mercury was in the methylated form. Neither of the two methods gives consistently higher results than the other, but both apparently are not extremely precise (R. F. Christensen, *personal communication*). However, both methods give high rates of recovery of standards (approximately 91% for atomic absorption and 96% for neutron activation).

Results and Discussion

EGGSHELL CHANGES

The major post-1946 eggshell changes encountered in this study involved great blue herons (−25%), red-breasted mergansers (−23%), common mergansers (−15%), and double-crested cormorants (−15%) in that order, all of these specimens collected in 1969 or 1970 from the Great Lakes States (Table 1). In this region, 9 of 13 species exhibited a statistically significant ($P < 0.05$) change in shell thickness or thickness index. However, for those species in which addled eggs were included, the sample is biased because these eggs have been incubated and the thinnest eggs would have been broken (11).

The heron eggs taken farther south displayed a generally smaller post-1946 eggshell change. While this change was statistically significant in 2 out of 7 species, eggs of the 7 southern species shown in Table 1 involved a mean (unweighted for sample size) change in thickness index amounting to −4%, whereas eggs of the four herons in the Great Lakes States showed a mean change of −11%. In eggs of the common egret, the only species for which we had samples from both Louisiana and the Great Lakes States, the thickness index change in Wisconsin was −7% ($P < 0.05$) and in Louisiana, −3% (not significant).

Eggs of each of the 19 species studied had a negative change in mean thickness index, although many of these changes were not statistically significant. Mean changes in measured thickness were negative in all but the pied-billed grebe. The mean thickness of one museum clutch of post-1946 Louisiana heron eggs from Texas was significantly greater than the pre-1947 mean. Changes in three museum clutches of Louisiana heron

eggs from Mexico were −13% ($P < 0.005$) in thickness and −12% ($P < 0.005$) in thickness index. The probability of negative changes in the 19 thickness-index estimates due to chance alone is infinitesimal ($P < 19 \times 10^{-7}$) and for negative changes in 18 of the 19 thickness estimates is very small ($P < 36 \times 10^{-6}$) (R. G. Heath, *personal communication*).

The differences between percent decrease in thickness and thickness index for great blue herons (Table 1) illustrate the value of the index. Thin-shelled great blue heron eggs have very rough shells which are difficult to measure accurately with a thickness micrometer; apparently, these eggs are also less dense. The thickness index takes into account both this change and the actual thickness, and probably is better related to the decrease in breaking strength that accompanies eggshell thinning.

In general, the consistent pattern of eggshell change in these species strongly suggests that, with larger post-1946 samples, statistically significant decreases in shell thickness will be found in virtually all fish-eating species of birds in these latitudes of North America. We are uncertain about the biological significance of decreases in shell thickness below 10%. Certainly, widespread eggshell breakage does not occur with changes below this magnitude. Normal eggshells do exhibit wide variation in thickness. Ernst Mayr (*personal communication*) feels that natural selection would produce an eggshell somewhat thicker than that normally needed to prevent breakage. Under normal conditions, the selection pressure against a genotype with too thin an eggshell is probably greater than that against a genotype which uses excess metabolic energy in producing a thick eggshell.

RESIDUE LEVELS IN EGGS

In Table 2, eggshell changes are compared with mean residues of organochlorines, PCB's, and mercury in the eggs. Means influenced by one high value are indicated by footnotes.

Residues of DDE and PCB's varied greatly, both among and within species. The herring gull eggs, collected by D. W. Anderson, from Sister Island in Green Bay had by far the highest levels of any species, while red-breasted merganser eggs collected 1 year earlier from the same island had almost 10 times less (Table 2). The levels of DDE and PCB's found in eggs from Louisiana were much lower than those in eggs from the Great Lakes States, with one exception, the green heron eggs from near Monroe, La. where DDT has been used extensively on cotton in the past (L.D. Newsom, *personal communication*). Eggs from Louisiana generally had less PCB's in relation to DDE than those from the Great Lakes States (see DDE: PCB ratios in Table 2).

TABLE 2.—Eggshell thickness changes and mean residues of organochlorines, PCB's, and mercury by species

SPECIES OF EGGS	COLLECTION LOCATION	NUMBER IN SAMPLE	PERCENT CHANGE IN SHELL THICKNESS	PERCENT CHANGE IN SHELL THICKNESS INDEX	MEAN RESIDUES IN PPM							RATIO OF DDE TO PCB'S
					PERCENT LIPID	DDE ¹	PCB'S ¹	DIELDRIN ¹	DDT + TDE ¹	BHC ¹	MERCURY ²	
GREAT LAKES												
Red-necked grebe	Rush Lake, Wis.	3	— 2.0	—13.0*	8.43	646.8	744.6	8.70	33.07	10.00	0.16	0.87
Pied-billed grebe	Rush and Mud Lakes, Wis.	9	+ 0.7	— 1.4	8.49	82.6	77.6	2.39	5.13	0.60	0.20	1.06
White pelican	W. Minnesota	5	— 7.3*	— 6.3	5.32	37.1	15.4	1.64	2.36	0.72	0.24	2.41
Double-crested cormorant	W. Minnesota	8	—14.0*	—15.1*	4.37	³ 188.4	132.2	7.07	4.98	15.4	0.43	³ 1.43
Great blue heron	Horicon Marsh, Wis.	5	—16.0*	—24.9*	4.83	472.1	175.3	10.10	44.27	0.79	0.29	2.69
Common egret	Horicon Marsh, Wis.	4	— 6.8	— 7.4*	6.24	³ 306.0	108.1	4.28	23.42	0.94	0.26	³ 2.83
Black-crowned night heron	Horicon Marsh, Wis.	6	— 5.2	— 9.7	6.68	81.6	70.2	9.37	6.07	0.76	0.48	1.16
American bittern	N. Wisconsin	1	0	— 3.3	7.27	6.1	12.2	0.21	0	0.14	0.15	0.50
Hooded merganser	N. Wisconsin	8	— 2.4	— 4.5	16.59	14.6	17.6	0.25	0.51	0.20	0.96	0.83
Common merganser	U.P., Mich., and N. Wis.	13	—15.8*	—15.3*	21.16	110.6	260.2	7.40	5.26	0.51	0.57	0.42
Red-breasted merganser	Green Bay, Lake Mich.	8	—17.0*	—22.9*	17.26	257.1	489.0	4.46	19.65	1.28	⁴ 1.59	0.59
Herring gull	Green Bay, Lake Mich.	10	—12.5*	—13.9*	7.63	2160.4	2224.5	13.01	33.27	7.64	⁵ 0.54	0.97
Black tern	Green Bay, Lake Mich.	5	—14.8*	—10.1*	9.65	50.7	89.7	2.56	2.98	0.20	0.49	0.57
Mean for Great Lakes			— 8.7	—11.4	9.53	339.5	339.7	5.50	13.92	3.01	0.49	1.25
LOUISIANA												
Green heron	Monroe	3	— 7.4	— 9.0*	6.69	209.7	11.0	0.88	31.63	0.12	⁶ 0.16	19.06
Light blue heron	Ferriday	5	— 7.1	— 4.4	6.13	39.2	13.5	4.56	5.51	0.10	⁶ 0.08	2.90
Cattle egret	Avery Island	5	— 3.4	— 3.6	6.50	26.0	28.0	5.66	1.86	0.17	⁶ 0.05	0.93
Common egret	Avery Island	5	— 1.0	— 3.4	4.77	46.3	³ 120.9	8.67	6.20	¹ 1.73	⁶ 0.09	³ 0.38
Snowy egret	Avery Island	5	⁷ — 5.5	⁷ — 5.3	6.49	24.6	24.7	1.06	1.02	0.53	⁶ 0.18	1.00
Louisiana heron	Avery Island and Ferriday	5	— 1.7	— 4.2	5.90	12.3	28.5	0.63	1.15	0.10	⁶ 0.09	0.43
Yellow-crowned night heron	Ferriday	5	— 1.7	— 0.7	5.85	52.5	18.6	0.18	13.95	0.36	⁶ 0.20	2.82
Mean for Louisiana			— 4.0	— 4.4	6.05	58.7	35.0	3.09	8.76	0.39	0.12	3.93

¹ Lipid-weight basis.² Wet-weight basis.³ Influenced by one high value.⁴ N = 3.⁵ N = 9.⁶ N = 1.⁷ Significant difference when post-1946 museum data are included.

* Significant difference (P<0.05) by Student's t-test.

Dieldrin residues were generally correlated with those of DDE ($r = 0.49$, $P < 0.001$), although the amounts were much lower than DDE and PCB's. Residue concentrations in eggs from Louisiana, however, were similar to those from the Great Lakes States. This perhaps reflects agricultural practices, since dieldrin is widely used to dress rice seed in Louisiana (L. D. Newsom, *personal communication*). As shown below, it apparently was important in eggshell thinning, at least in eggs of most species from the Great Lakes region.

Residues of DDT + TDE were correlated with DDE similar to dieldrin ($r = 0.44$, $P < 0.001$). This is expected since the DDE and TDE are degradation products of DDT. The levels of DDT + TDE were of the same order of magnitude as dieldrin and those in eggs from Louisiana were again similar to those from the Great Lakes States.

BHC was present in higher concentrations in eggs from the Great Lakes States, just as DDE was. The reason for this difference is not readily apparent since BHC is used in agriculture in both areas.

Mercury values for the various species (Table 3) are expressed in ppm on a wet-weight rather than lipid-

weight basis because mercury is distributed more or less evenly throughout the egg and is apparently not lipophilic as are the chlorinated hydrocarbons. For those species with all eggs containing less than 0.25 ppm of mercury (American bittern, pied-billed grebe, white pelican, red-necked grebe, and all seven species of herons from Louisiana), the residues may represent background levels.

Of all eggs analyzed for mercury in this study, 29% had at least 0.5 ppm and 9% at least 1.0 ppm. Nine of the 13 species from the Great Lakes States had one or more values above the 0.5-ppm level. Fimreite (12) fed grain treated with a mercurial fungicide to pheasants; these birds, when sacrificed, had 3-13 ppm mercury in their livers, and their eggs with 0.5 to 1.5 ppm suffered greater embryonic mortality than controls. Vermeer (31), however, sampled one egg in each of 28 successful herring gull nests and found 24 to contain mercury levels between 0.5 and 2.0 ppm. The significance of our mercury data is thus unknown at this time.

Eggs of the three merganser species had relatively high levels of mercury, with one clutch of inviable hooded merganser eggs having > 2 ppm. These were also the only species for which abandoned clutches, dump nests, or un-

TABLE 3.—Distribution of mercury residues in eggs

SPECIES OF EGGS	AREA OF COLLECTION	NUMBER OF EGGS	NUMBER OF EGGS PER RESIDUE LEVEL (PPM, WET-WEIGHT BASIS)				
			<0.05	0.05- 0.24	0.25- 0.49	0.50- 0.99	>1.0
Red-necked grebe	Rush Lake, Wis.	3		3			
Pied-billed grebe	Mud and Rush Lakes, Wis.	9		7	2		
White pelican	Milan, Minn. (western)	5		2	3		
Double-crested cormorant	Milan, Minn. (western)	8		1	3	4	
Great blue heron	Horicon Marsh, Wis.	5		3	1	1	
Common egret	Horicon Marsh, Wis.	4	1	1	1	1	
Black-crowned night heron	Horicon Marsh, Wis.	6		3	1	1	1
American bittern	Ladysmith, Wis.	1		1			
Hooded merganser	Chippewa Co., Wis.	6		2	2	2	
Hooded merganser	Price Co., Wis.	2					2
Common merganser	Brule & Pine Rivers, Wis.	2			1		1
Common merganser	Seney, Mich. (U.P.)	11			8	2	1
Red-breasted merganser	Sister Island, Green Bay, Wis.	3					3
Herring gull	Sister Island, Green Bay, Wis.	9			5	4	
Black tern	Oconto, Wis. (on Green Bay)	5			3	2	
Seven species of herons and egrets	Louisiana	7		7			
TOTAL		86	1	30	30	17	8

¹ Four of these were reported as > 1.0 ppm, but had to be adjusted for desiccation.

hatched eggs were found in the course of our searching for active nests. Of 16 nests located for the three species of mergansers, 11 were in one or another of these categories. However, some eggs with more than 1.0 ppm came from active nests and were in an advanced incubation stage when taken. The addled red-breasted merganser eggs (Table 4) showed high mercury levels (1.1-1.9 ppm) and also high dieldrin levels (6.44-9.73 ppm, lipid basis.), while viable eggs collected from the same nests had much lower dieldrin levels (0.15-2.37 ppm). Viable eggs were not analyzed for mercury. The comparison of means of dieldrin did not show a significant difference. The sum of DDT and TDE was also higher in addled than in viable eggs. However, the combination of aldrin (the parent compound of dieldrin) and mercury is apparently more toxic to birds than is mercury alone (5, 6, 8). These data suggest a need for research into the relationship between dieldrin and other chemicals and embryonic mortality in this species.

Some of our highest mercury values were from eggs collected in the Chequamegon National Forest and Nicolet National Forest in northern Wisconsin, and from the Seney National Wildlife Refuge in Michigan. Mercury values in eggs of herring gulls and black terns collected from Green Bay, Lake Michigan, were also somewhat higher than in most eggs from other areas. Eggs from other areas showed no consistent differences, except that levels in eggs from Louisiana were consistently low.

CONTRIBUTIONS OF RESIDUES TO EGGSHELL THINNING

Both DDE and dieldrin have been associated with eggshell thinning (2, 7, 22). However, as our data show, an egg with high levels of one type of chlorinated hydrocarbon will often have high amounts of the others as

well. Therefore, spurious correlation is a possibility, and in our data, this was apparently the case. In simple correlation, all of the residues had significantly negative coefficients with percent of pre-1947 thickness index in one category or another when we combined the data into family groups. We, therefore, used partial correlation (29) to segregate the spurious correlations and determine the residues which were significantly related to eggshell thinning. In order for the analysis to be valid, it was necessary to assume that the samples were collected in random fashion and that the transformed data were normally distributed. Our data essentially satisfied both requirements. Since the sample of herring gull eggs was not a random one and also because the distribution of residue values was very different from those of any of the other species, these data were excluded from all analyses of combined groups. All residue data were transformed to their logarithms for the correlations because this gave a more normal distribution of residue values. Virtually all correlation coefficients were increased when the log transformation was used. Partial correlation coefficients between percent change in pre-1947 thickness index and residues were calculated for all of the chemicals which were significantly correlated with percent thickness index in the simple correlations.

Our data did not satisfy the theoretical requirements for multiple-regression analysis, but we did calculate the coefficient of determination, R^2 , to show the amount of variation in percent pre-1947 thickness index which could be accounted for by the combined residues.

The extent of correlation of a given residue type with the thickness index varied from family to family (Table 5). Sample sizes for most species were too small to permit meaningful intraspecific analyses by partial correlation.

TABLE 4.—Residues and eggshell parameters for three viable (V) and three addled (A) eggs from the same nests of red-breasted mergansers

NEST	TYPE OF EGG	RESIDUES IN PPM ¹						PERCENT LIPID	EGGSHELL THICKNESS	EGGSHELL THICKNESS INDEX
		DDE	PCB's	DIELDRIN	DDT+TDE	BHC	MERCURY			
1	V	187	224	2.37	10.5	0.69	(²)	15.6	0.33	1.74
	A	336	294	9.73	43.7	0.85	1.1	18.8	0.34	1.72
2	V	201	607	0.15	19.0	0.90	(²)	16.8	0.28	1.51
	A	330	348	8.31	21.6	0.60	1.8	16.6	0.30	1.48
3	V	351	1,375	1.98	0	1.37	(²)	12.6	0.28	1.43
	A	328	644	6.44	27.7	5.06	1.90	25.3	0.29	1.55
MEAN	V	246.0	735.6	³ 1.50	9.86	0.99		15.0	0.30	1.56
	A	297.6	428.7	³ 8.16	31.0	2.17	1.6	20.2	0.31	1.58

¹ Residues on lipid basis for organochlorines, on wet-weight basis for mercury.

² Not analyzed for.

³ Means significantly different ($P < 0.01$) when calculated on wet-weight basis.

TABLE 5.—Coefficients of determination (R^2) and partial correlation coefficients for correlation of percent changes in shell-thickness index with the logarithm of each residue

	NUMBER OF EGGS	R^2	DDE	PCB's	DIELDRIN	DDT + TDE	BHC	MERCURY
Eggs in which mercury was measured:								
Heron eggs	23	.543***	-.448*	-.224	-.196	+.333	—	—
Merganser eggs	24	.646***	+.100	-.205	-.241	+.102	-.175	-.410
All eggs in Upper Great Lakes States (12 species)	69	.392***	-.170	-.019	-.222	+.032	—	-.299*
All added eggs (7 species)	30	.204*	—	—	—	—	—	-.451*
All viable eggs (16 species)	46	.505***	-.115	-.188	-.369*	+.032	—	—
Both viable and added eggs (18 species)	76	.367***	-.126	-.039	-.242*	+.040	—	-.253*
Eggs without mercury considered:								
Heron eggs	49	.494***	-.575***	-.199	+.159	+.359*	—	—
Merganser eggs	29	.609***	+.048	-.410*	+.115	-.062	-.199	—
All eggs in Upper Great Lakes States (12 species)	74	.352***	-.225	-.174	-.097	+.063	—	—
All viable eggs (16 species)	77	.470***	-.384***	-.284*	-.034	+.131	+.078	—
Both viable and added eggs (18 species)	107	.378***	-.335***	-.251*	-.003	+.122	+.157	—

* $P = < 0.05$.

** $P = < 0.01$.

*** $P = < 0.005$.

It appears, however, that there may be very important differences between families in the effect of a given compound on eggshell thinning. For the herons, DDE was the only compound which was significantly correlated with percent of pre-1947 thickness index. DDT + TDE showed a significantly positive correlation with percent of pre-1947 thickness index in the herons; however, this may be an artifact of the number of correlations performed, a statistically significant relationship occurring by chance alone. Previous field studies have failed to show that DDT or TDE was a significant factor in eggshell thinning; Jefferies (17) reported that DDT apparently increased eggshell weight in Bengalese finches, but this increase was very small (less than 1%) and probably not significant. For mergansers, PCB was the only compound among DDE, PCB, dieldrin, DDT + TDE, and BHC which was significantly correlated with percent of pre-1947 thickness index. Although the sample size was small ($N=11$), the six residue types together accounted for 97.6% ($P < 0.005$) of the variation in percent thickness index in the grebes. However, none of the individual residues was significantly correlated with percent of pre-1947 thickness index in simple correlation, and so we did not calculate partial correlation coefficients. For the other families and individual species, sample sizes were small, and the coefficient of

determination did not show a significant reduction in variance of the percent thickness index.

There are several theories concerning the physiological mechanism of eggshell thinning due to pesticides (17, 26). All have a common problem: they do not fully explain why there should be interfamilial or interspecific differences in susceptibility to DDE-caused eggshell thinning. However, there do seem to be real differences, at least between such species as Japanese quail (*Coturnix coturnix*) and mallards (*Anas platyrhynchos*) (15). Since the species we studied are related in their food habits (all except the cattle egret feed on aquatic organisms, and most feed mainly on fish), we performed partial correlation analyses on the combined data, but with the herring gull data excluded. The results of these analyses (Table 5) should be viewed with caution, since they represent combined samples. The simple correlation of mercury with percent thickness index for added eggs apparently carries over to the larger groups in which both added and viable eggs were combined. When only viable eggs were considered, DDE, dieldrin, or PCB was the most important, and mercury was not correlated at all. The results do not mean that any of the residues which had significant partial correlation coefficients are importantly related

to eggshell thinning in all of the species in the sample. Indeed, it is evident that this was not the case. However, the results do indicate that these residues were important to many of the species in the sample. Thus, we consider that both DDE and dieldrin are important to many species, especially herons, and PCB is important to mergansers and perhaps some other species as well in respect to eggshell thinning. It appears that DDT, TDE, and BHC have very little relation to shell thinning. The importance of mercury is puzzling, however, since its significance occurred in addled eggs but not in viable eggs. Larger sample sizes are needed for individual partial correlation analysis of each of the species studied here.

We noted consistently high correlations ($P < 0.001$) between DDE and PCB's in all of our samples. This may be the result of similar storage characteristics in fat, similar distribution patterns in ecosystems, or causal relationships in fat storage. With regard to causal relationships in fat storage, Lichtenstein (23) reported increased mortality of houseflies when both DDT and PCB's were present as opposed to DDT alone, and Street (30) demonstrated a negative effect of DDT fed to rats on storage of dieldrin. However, in our samples there was a significantly positive correlation between dieldrin and DDT + TDE, suggesting little or no negative effect of DDT + TDE on dieldrin levels. Heath *et al.* (15) found no synergism between DDE and PCB's in toxicity tests on Japanese quail, but there remains a great deal to be learned of the relationships between PCB's and the other organochlorines. It is entirely possible that no causal relationships are involved, and that the high correlations observed are due solely to similar distribution patterns of the organochlorines in ecosystems.

All but 3 of the 34 simple correlation coefficients which we determined for DDE were negative, while only 24 were negative for dieldrin. We therefore feel that DDE significantly affects many species, but that the residue concentrations needed to cause a given degree of eggshell thinning vary greatly among species. For example, herring gull eggs had approximately 40 times as much DDE as black tern eggs, yet the mean change in thickness index was only 4% greater for herring gulls. Differences such as these, even when log transformations are used, reduce the magnitude of the correlation coefficient for the combined data, although DDE may cause eggshell thinning in both species.

In most cases, the R^2 values (Table 5) were rather low, although many were significant by the F -test. These chemicals are by no means the only ones to which birds are subjected in greater amounts today than in former times. Many natural environmental and physiological

factors such as heat, humidity, and nutrition can affect eggshell thickness (27). Variation due to these other factors may mask the effects which we attempted to measure. However, it is clear that DDE and dieldrin are importantly related to eggshell thinning in many species, and that PCB's are similarly related to eggshell thinning in mergansers.

When we compared the mean change in thickness index to the mean of the sum of all organochlorines for each species, a trend toward a linear relationship on a log-log basis was evident (Fig. 1). However, the yellow-crowned night heron and pied-billed grebe data did not appear to fit the line. No statistical analysis of this relationship was possible because of differences in sample sizes.

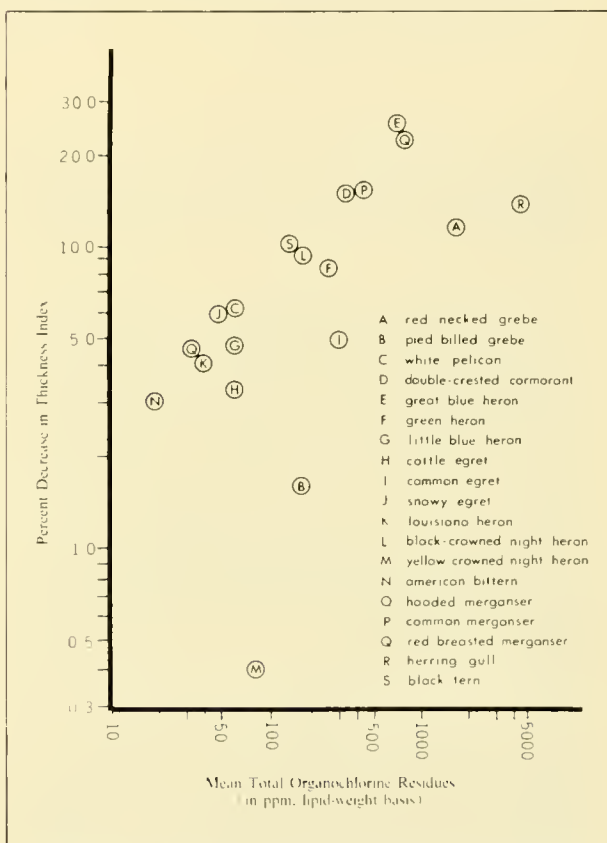


FIGURE 1.—Mean shell-thickness index changes and mean residue levels for each species (log-log basis)

See Appendix for chemical names of compounds discussed in this paper.

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International Cooperative Study of Organochlorine and Mercury Residues in Wildlife, 1969-71

A. V. Holden¹

ABSTRACT

A two-part collaborative study of organochlorine pesticides, polychlorinated biphenyls (PCB's), and mercury residues was carried out by 26 laboratories in 12 countries. The first part involved the analysis of three test samples containing organochlorine residues and one group of test samples containing mercury. One organochlorine sample contained seven pesticides or derivatives added to corn oil; a second was a standard solution of a PCB formulation in hexane; and the third a homogenate of cormorant (*Phalacrocorax carbo*) muscle containing mainly PCB's. With few exceptions, agreement among the analysts involved was reasonably good and acceptable for monitoring wildlife residues. Coefficients of variation for the various organochlorines were from $\pm 10\%$ to $\pm 17\%$. The test samples containing mercury included a group of freeze-dried homogenates of the muscle tissue of pike (*Esox lucius*) and an ampoule of methylmercury dicyandiamide in water; the samples were analyzed for total and methylmercury. Again, agreement was reasonably good, although only four laboratories reported total mercury values and eight, methylmercury values.

The second part of the program required the sampling and analysis of several species of wildlife from both terrestrial and aquatic environments, including fish, shellfish, and the eggs of birds. Samples were taken in specified numbers and at specified times, from both areas considered to be free of any pesticide usage and areas known or believed to be seriously polluted. Eggshell thickness indices were also determined. The results of the wildlife analyses demonstrated the difficulties in selecting species appropriate for international monitoring programs, in identifying (before analysis) areas of high contamination, and in relating concentrations to measurable biological effects. Samples of any one species from areas regarded as unpolluted in different countries contained a wide range of organochlorine concentrations. The PCB levels in mussels from such areas contained 0.01 to 0.67 mg/kg, and DDE levels in herring ranged

from <0.01 to 0.30 mg/kg. The differences between residue results for samples in this second part of the study were much greater than the analytical variations estimated from the first part of the study.

Introduction

A series of voluntary international cooperative studies of residues of pesticides and other contaminants in the environment has resulted from a meeting sponsored by the Organization for Economic Cooperation and Development (O.E.C.D.) held in Paris in 1966. A report on the second study (1967/1968), published in an earlier issue of this Journal (1), was presented at the Second O.E.C.D. Technical Meeting on Pesticides in the Environment, held in the Netherlands in 1969; also at that meeting, a further study was designed for the period 1969-71. The findings of this third study are reported here.

At the original meeting in Paris, proposals for an international monitoring program were made, but most countries were unprepared for such work. Since that time, interest in the subject of environmental pollution has increased, and a recommendation for global monitoring was passed at the United Nations Conference on the Human Environment in Stockholm in 1972. For the monitoring of organochlorine residues using indicator species, it is essential that all laboratories engaged in the work be able to reach agreement, both qualitatively and quantitatively, on the residues present; suitable exchange samples provide a means for assessing such agreement.

The species selected for monitoring must be capable of providing an adequate number of samples for the calculation of the mean contamination level of each population studied and must be sufficiently abundant to allow

¹ Freshwater Fisheries Laboratory, Pitlochry, Scotland (acting as coordinator).

adequate sampling for analytical purposes without endangering the population. Adequate knowledge must also be available beforehand on the variation of residue levels between individuals of the population, so that the number of individuals taken for analysis will provide a mean value of the contamination level of the population, of sufficient accuracy for the statistical evaluation of differences between populations or between successive sampling occasions. For the final assessment of the biological significance of a particular residue, more extensive knowledge of the effects of residues at various contamination levels is required than exists at the present time.

In the second collaborative study (1), the agreement among analysts on the presence and concentrations of dieldrin, DDE, TDE, and DDT in exchange samples was reasonably good only for material which required the minimum of processing prior to gas-liquid chromatographic (GLC) analysis. With such samples, the coefficient of variation was $\pm 25-30\%$. For more difficult samples, the coefficient of variation was $\pm 30-60\%$. No allowance for or determination of polychlorinated biphenyls (PCB's) was made. In addition, distribution of residues among individuals of most populations of starlings and pike was found to be skew. For the detection of a 25% difference in contamination levels, it was calculated that 50 individuals would be required; for a 75% difference, 10 individuals were sufficient.

The third study included analysis for organochlorines and extended the analytical requirements to PCB's and mercury, and appropriate exchange samples were circulated. The wildlife samples, both freshwater and marine, included fish and fish-eating birds, sampled according to a detailed program. The second study had been confined to areas believed to be uncontaminated by direct application or disposal of pesticides, but in this third study both uncontaminated and contaminated areas were selected for sampling, in the hope of establishing the existence of differences between contamination levels. In addition, where the eggs of certain species of birds were to be sampled, measurements of eggshell thickness indices were requested in an attempt to correlate these with organochlorine residue levels. In all, 26 laboratories in 12 countries took part in this third study; these laboratories are identified in Table 1.

A total of 18 laboratories participated in the analytical program, 17 in the nine member countries of O.E.C.D., and in addition the analytical laboratory of Euratom at the European Community Commission Joint Research Center in Ispra, Italy. Most of the laboratories had taken part in the second study. Since the second study, analytical methods have been developed for the separation and measurement of PCB's; therefore, it was an-

TABLE 1.—*Laboratories participating in international cooperative study of organochlorine and mercury residues in wildlife, 1969-71*

COUNTRY	PARTICIPATING LABORATORIES
Canada	Ontario Research Foundation (ORF), Sheridan Park, Ontario, Canada. Fisheries Research Board of Canada (FRB), St. Andrews, New Brunswick, Canada.
Denmark	Veterinary and Agricultural College, Department of Pharmacology and Toxicology, 13 Bülowsvej, Copenhagen V, Denmark.
Euratom (Italy)	Analytical Laboratory, Euratom, European Community Commission Joint Research Center, Ispra, Italy.
Finland	State Institute of Agricultural Chemistry (Agric.), 00170 Helsinki, Finland. State Veterinary Medical Institute (Vet. Med.), 00550 Helsinki, Finland. State Game Research Institute, 00170 Helsinki, Finland. Reactor Laboratory, State Institute for Technical Research, Otaniemi, Finland.
Germany	Department of Ecological Chemistry, 800 Munich 15, Landwehrstrasse 61, Federal Republic of Germany. Federal Institute for Fisheries Research, 2 Hamburg 50, Palmallee 9, Federal Republic of Germany.
Netherlands	Institute of Veterinary Pharmacology and Toxicology (Vet. Tox.), University of Utrecht, Utrecht, Netherlands. Central Institute for Nutrition and Food Research (CIVO), Utrechtseweg 48, Zeist, Netherlands. National Institute of Public Health (RIV), Sterrenbos 1, Utrecht, Netherlands.
Norway	Veterinary College of Norway, Department of Pharmacology, Oslo 4, Norway. Agricultural College of Norway, Department of Zoology, Vollebek, Norway.
Portugal	Laboratory of Phytopharmacology, Quinto do Marques, Oeiras, Portugal.
Spain	Institute of General Organic Chemistry, Juan de la Cierva 3, Madrid-6, Spain.
Sweden	Special Analytical Laboratory, Swedish Environment Protection Board, Stockholm, Sweden. Swedish Museum of Natural History, Stockholm, Sweden. Royal Institute of Technology, Department of Nuclear Chemistry, Stockholm, Sweden.
United Kingdom	Laboratory of the Government Chemist (LGC), Cornwall House, Stamford Street, London SE1 9NQ, U.K. Ministry of Agriculture, Fisheries and Food (MAFF), Fisheries Laboratory, Brunham-on-Crouch, Essex, U.K. Nature Conservancy (NC), Monks Wood Experimental Station, Abbots Ripton, Huntingdonshire, U.K. Department of Agriculture and Fisheries for Scotland, Freshwater Fisheries Laboratory (FFL), Pitlochry, Perthshire, Scotland.
United States	U.S. Department of the Interior (USDI), Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Md. 20810, U.S.A. U.S. Environmental Protection Agency (EPA), Gulf Breeze Laboratory, Gulf Breeze, Fla. 32561, U.S.A.

anticipated that the measure of agreement among laboratories in respect to the organochlorines would be improved. Several laboratories were also engaged in mercury analysis.

Interlaboratory Quality Evaluation Program for Organochlorine and Mercury Residue Analyses

DESCRIPTION OF TEST SAMPLES

Four types of test samples were circulated among the participating laboratories, for analysis by the methods each currently used. An outline of the methods used for

organochlorine analysis is given in Table 2; the techniques involved in the analysis of the mercury check samples are discussed later.

Sample No. 1—a mixture of seven organochlorine insecticides or derivatives in corn oil together with a sample of the unspiked oil.

This sample was distributed by Mr. W. L. Reichel, Patuxent Wildlife Research Center, Laurel, Md., U.S.A. On receipt of the report from each laboratory, the correct analysis for the added residues was provided.

TABLE 2.—Analytical techniques for organochlorine analysis

LABORATORY	METHOD OF EXTRACTION ¹	CLEANUP	PRE-GLC SEPARATION	GLC PACKING
Canada				
ORF	ether-hexane (1:1)	Carbon-Celite at -70°	Florisil	4% SE-30/6% QF-1
FRB	hexane	Alumina	Silica	4% SE-30
Denmark	ethanol-hexane (3:1, cold), then 10% ether in petroleum ether (cold)	Fuming H ₂ SO ₄		SF-96/QF-1
Euratom	acetonitrile and petroleum ether	Florisil	Florisil	1) 3% DEGS 2) 5% DC-200/7.5% QF-1 3) 1.5% OV-17/1.95% QF-1
Finland				
Agric.	hexane or acetone-hexane (1:9)	DMF/hexane or alumina	Silica	1) Apiezon L 2) E. glycol adipate
Vet. Med.	ether on column (cold)	H ₂ SO ₄ or TLC	TLC	1.25% SF-96/3.75% QF-1
Germany				
Munich	benzene	Alumina		3% OV-1
Hamburg	hexane	Alumina	Silica	1) DC-200 2) DC-200/QF-1
Netherlands				
Vet. Tox.	hexane	Alumina	Silica	1) DC-200 2) QF-1
CIVO	hexane	Alumina	Silica	1) 3% OV-1 2) 1.8% OV-1/2.7% QF-1
Norway	ether on column (cold)	H ₂ SO ₄ or alcoholic KOH		1) 10% QF-1 2) 4% SF-96
Portugal	hexane	DMF/hexane, alumina		1) 5% QF-1 2) 4% SE-30/6% QF-1
Spain	hexane/acetone (4:6)	CH ₃ N/hexane	Florisil	1) 9% DC-200 2) 5% DC-200/7.5% QF-1
Sweden	hexane	1) Silica 2) KOH 3) fuming H ₂ SO ₄		1) SF-96 2) 1.75% SF-96/3.75% QF-1
United Kingdom				
LGC	acetone/hexane (1:2)	DMF/hexane, alumina	Silica	1) SE-52 2) Apiezon L
FFL	hexane	Alumina	Silica	1) 6% DC-200 2) 4% DC-200/6% QF-1
United States				
USDI	hexane	CH ₃ CN/hexane	Florisil, Tl.C	1) 3% OV-1 2) 3% XE-60
EPA	petroleum ether	CH ₃ CN/hexane	Florisil	1) 3% DC-200 2) 5% QF-1 3) 1.5% DC-200/2.5% QF-1

¹ Hot extraction, unless otherwise indicated.

enabling the laboratories to check for errors in standards or technique before proceeding with the analyses of wildlife samples.

Sample No. 2—an ampoule containing a solution of a 60% technical PCB mixture (Clophen A.-60).

This sample was distributed by Dr. J. H. Koeman, Institute of Veterinary Pharmacology and Toxicology, Utrecht, Netherlands. After laboratories reported their values for the PCB content of this solution, analysts were informed of the true concentration.

Sample No. 3—a homogenate of the total body (less bill, legs, and feathers) of a cormorant (Phalacrocorax carbo) found dead in the Netherlands in 1970. The weight of fresh tissue (ground with anhydrous sodium sulphate) was given with the sample.

This sample was primarily for PCB analysis, although many laboratories examined it for other organochlorine residues as well. It was distributed by Dr. J. H. Koeman, Utrecht, Netherlands.

Sample No. 4—a group of freeze-dried homogenates of the muscle tissue of pike (Esox lucius), and an ampoule of methylmercury dicyandiamide in water.

These samples were for the analysis of mercury in both methyl and total forms and required the addition of a specified amount of water before analysis to reconstitute the tissue. Two specimens (A and B) were identical, but to one (B) was added to contents of the ampoule before analysis. A third tissue sample of high mercury content (C) was analyzed separately. A fourth tissue sample (D) was included for experience in analyzing this type of sample, but results were not evaluated. All results were recorded on a wet-weight basis, and the difference between specimens A and B calculated. The samples were distributed by Dr. S. Jensen, Special Analytical Laboratory, Lanthrukshögskolan, Uppsala, Sweden.

ANALYTICAL PROCEDURES (TEST SAMPLES)

The methods used for the analysis of organochlorines varied widely (Table 2), and sometimes techniques differed for the corn oil and cormorant samples. For the latter, extraction solvents included ether, hexane, hexane/acetonitrile, hexane/acetone, and hexane/acetonitrile/methylene chloride. Fewer analysts used a solvent partition cleanup than an adsorbent column process (usually Florisil or alumina), while separation of PCB's from other organochlorines was usually achieved with silica columns. A wide variety of stationary phases was used in the GLC analysis, including SE-30, SF-96, DC-200, OV-1, OV-17, QF-1, XE-60,

Apiezon L, DEGS, and mixtures of QF-1 with other nonpolar phases. No two laboratories used the same complete procedure for extraction, cleanup, and GLC analysis.

For the calculation of PCB concentrations, a wide variety of methods was also used. Many analysts used peak heights, with the number of peaks varying from one to six. Others used calculations of peak area: by electronic integration using nine peaks; by a total area method involving cutting and weighing; or, in some cases, by the equivalent of area measurement involving the product of peak height and relative retention time. One laboratory measured the optical density on thin layer plates. The reference solutions employed were usually 50% or 60% chlorinated formulations, but one laboratory based its measurements on DDE or dieldrin standards which had previously been calibrated with reference to a PCB formulation.

The analytical methods for mercury residues are referred to later.

DISCUSSION OF RESULTS (TEST SAMPLES)

The corn oil sample (No. 1) was analyzed by more laboratories (17) than the other samples, with 2 laboratories using two separate methods, giving a total of 19 sets of results. Fourteen laboratories analyzed the PCB solution (No. 2); 14 analyzed the cormorant sample (No. 3) with 3 of them using more than one method, giving a total of 18 sets of results for the PCB concentration. The mercury samples (No. 4) were examined by nine laboratories, some doing only methyl or total mercury analyses. Several laboratories, due to lack of experience, reported difficulty in the analyses for methylmercury.

Sample No. 1

Although this sample contained seven different organochlorine residues, most laboratories reported only six, being unable to identify or quantify dichlorobenzophenone (DCBP). The results of these analyses are given in Table 3. One laboratory in the United Kingdom (LGC Laboratory) used two different GLC columns for separate analyses, and one laboratory in Finland (Agric.) used different processing techniques for separate analyses. One laboratory in West Germany (Hamburg) reported only three of the seven residues present and estimated dieldrin approximately; this dieldrin value was not included in subsequent calculations. The same laboratory was unable to estimate heptachlor epoxide or *o,p'*-DDT.

An examination of the distribution of the values recorded for the individual residues suggests that the values for dieldrin, *o,p'*-DDT, and *p,p'*-DDT are normally distrib-

TABLE 3.—Analyses of test sample no. 1—spiked corn oil

LABORATORY	CONCENTRATIONS IN MG/KG						
	HEPTACHLOR EPOXIDE	<i>p,p'</i> -DDE	<i>p,p'</i> -TDE	<i>p,p'</i> -DDT	<i>o,p'</i> -DDT	DIELDRIN	DCBP
Canada							
FRB	3.3	11.9	5.7	3.8	1.1	4.0	—
ORF	3.5	12.0	5.9	4.0	2.5	4.7	1.9
Denmark	3.2	13.5	6.0	4.1	3.5	3.0	—
Euratom	4.2	13.8	7.5	4.5	3.5	4.9	1.9
Finland							
Agric., (two methods)	4.0	15.1	6.7	4.9	4.1	4.9	—
Vet. Med.	4.0	15.3	6.9	5.0	4.0	5.0	—
	3.4	13.1	5.9	4.4	3.6	4.2	—
Germany							
Hamburg	—	11.1	6.1	3.4	—	¹ 1.9	—
Munich	4.8	10.3	9.6	5.0	3.2	5.0	—
Netherlands							
CIVO	4.3	14.8	7.0	4.4	4.2	5.2	—
RIV	3.3	9.7	6.3	3.6	—	3.9	—
Vet. Tox.	3.0	11.7	6.6	3.1	3.7	4.3	—
Norway	2.7	16.5	7.9	5.5	3.7	6.0	—
Spain	4.8	14.2	3.6	3.4	2.0	3.1	—
United Kingdom							
FEL	4.2	15.3	6.7	4.1	3.6	4.5	1.8
LGC (two methods)	3.4	11.7	6.7	3.7	2.9	4.6	—
	3.2	11.8	7.0	3.7	2.7	4.6	—
United States							
EPA	4.0	14.7	7.6	5.2	3.5	5.0	—
USD1	3.0	14.2	6.1	4.6	3.2	4.4	1.7
True Spike Values	4.0	15.0	7.5	4.5	3.5	5.0	2.0

NOTE: — = no value reported.

¹ Approximate estimate only; value not included in subsequent calculations.

uted; however, there is some doubt about the distribution of the values for *p,p'*-TDE, *p,p'*-DDE, and, in particular, heptachlor epoxide. The results for heptachlor epoxide appear to fall into two groups, one around 3.2 mg/kg and the other around 4.1 mg/kg. The statistical analyses of the data, referred to in the following paragraphs and summarized in Table 4, were based on the assumption that distribution is in fact normal. The mean values for the residues found were in all instances below the true values, suggesting that some loss of residues in processing was experienced by most laboratories. The recoveries ranged from 87.8% for *p,p'*-DDE to 96.2% for *o,p'*-DDT. Yet, of the six residues which were identified by most analysts, two laboratories obtained values within $\pm 5\%$ of the true values for five of these, and three were within $\pm 10\%$ for five residues.

At the level of estimation of the residues in the spiked sample, most laboratories reported that they did not find any residues in the unspiked corn oil. Generally, a recovery of at least 90% of all residues in this sample was possible, but no relationship between the methods used for extraction or cleanup and the efficiency of recovery

could be found (Tables 3 and 4). The laboratory of the State Institute of Agricultural Chemistry, Helsinki, compared the alternative cleanup techniques of de Faubert Maunder *et al.* (2) and Holden and Marsden (3), and obtained remarkably similar results. The laboratory also used two different stationary phases (Apiezon L and ethylene glycol adipate) in two instruments, several residues being estimated on both instruments, and each being determined by triplicate analyses; these mean residue values are given in Table 3. The results suggest that the differences between analysts do not necessarily lie in the procedures adopted for cleanup or GLC analysis, but may be due to variation in standard solutions or in the methods of preparation of the initial extract.

Overall, the results from this sample suggest that there has been an improvement in the agreement among analysts since the second study in 1967/1968 (1). The coefficients of variation for five organochlorine residues obtained for two samples in the second study (1) and for the corn oil sample in the present study are given in Table 5. Both the chicken egg homogenate sample of 1967/1968 (prepared from chickens fed with a

TABLE 4.—Summary of analyses of test sample no. 1—spiked corn oil

COMPOUND	NUMBER OF DETERMINATIONS	CONCENTRATIONS IN MG/KG				COEFFICIENT OF VARIATION (PERCENT)	PROPORTION WITHIN $\pm 10\%$ OF MEAN	PERCENT RECOVERY BASED ON TRUE CONCENTRATION
		TRUE CONCENTRATION	RANGE OF REPORTED VALUES	MEANS OF ANALYSES	STANDARD DEVIATION			
Heptachlor epoxide	18	4.0	2.7- 4.8	3.68	± 0.62	± 16.8	8 in 18	92.0
Dieldrin	¹ 18	5.0	3.0- 6.0	4.52	± 0.72	± 15.9	12 in 18	90.4
<i>p,p'</i> -DDE	19	15.0	9.7-16.5	13.20	± 1.90	± 14.4	7 in 19	87.8
<i>p,p'</i> -TDE	² 17	7.5	5.7- 7.9	6.62	± 0.65	± 9.8	11 in 17	88.3
<i>p,p'</i> -DDT	19	4.5	3.1- 5.5	4.23	± 0.68	± 16.1	8 in 19	94.0
<i>o,p'</i> -DDT	³ 16	3.5	2.0- 4.2	3.37	± 0.60	± 17.7	9 in 16	96.2
DCBP	4	2.0	1.7- 1.9	1.82	—	—	4 in 4	91.0

¹ When two abnormally low values from Denmark and Spain were excluded (values less than 3 times the standard deviation), the range was 3.9-6.0 mg/kg with a mean recovery of 93.7%.

² Excludes one unusually low value from Spain and one high value from West Germany (Munich), values differing from the mean by more than 4 times the standard deviation.

³ Excludes one low value from Canada (FRB), a value less than the mean by 3.8 times the standard deviation.

pesticide mixture) and the spiked corn oil sample of this study required a significant amount of processing, including cleanup and in many cases separation of residues prior to GLC analysis. Nevertheless, the results for the spiked corn oil are distinctly better than those obtained for the chicken egg in the previous study, although understandably not as good as the values for the standard solution in hexane which required no more than dilution and direct injection for GLC analysis. Coefficients of variation in the range $\pm 10\%$ to $\pm 15\%$ for different organochlorine residues in samples requiring complete processing are perhaps the best that can be expected at the present time.

Sample No. 2 (PCB solution)

This sample, an ampoule of a solution of Clophen A.-60 in hexane (a 60% chlorinated PCB), was analyzed for comparing it with a solution of an appropriate PCB standard chosen by the analyst (Table 6). The true value given subsequent to the analysis being reported was 9.8 mg/liter of Clophen A.-60. Fourteen laboratories reported, two giving estimates by two different methods. Twelve results were obtained using 60% chlorinated PCB standards, the range of values being 8.4 - 12.6 mg/liter, the mean 10.17 mg/liter, and the coefficient of variation $\pm 10.2\%$. Two of the three laboratories using Clophen A.-60 reported a concentration of exactly 10.0 mg/liter; two of three using Clophen A.-50 as reference estimated the concentration as 8.0 mg/liter. One laboratory using Phenoclor DP.6 obtained a value of 12.6 mg/liter. Seven results based on Aroclor 1260 gave a range of 9.0 to 11.4 mg/liter with a mean of 9.97 mg/liter (coefficient of variation $\pm 9.7\%$). One

laboratory obtained two estimates of 9.6 and 11.0 mg/liter with two different types of GLC column. By comparison, the coefficients of variation for the results of analyses for six different organochlorine pesticides in corn oil (Sample No. 1) ranged from $\pm 9.8\%$ to $\pm 16.8\%$.

For this commercial PCB mixture, the agreement among the analysts using appropriate reference solutions was very satisfactory despite the wide variety of methods of calculation; however, there seems to have been some variation due to the different commercial products used as reference standards.

Sample No. 3 (cormorant)

The analysis of the homogenate of cormorant tissue involved extraction, cleanup, and, where possible, the separation of PCB residues from other organochlorines. In addition to the PCB residues, high concentrations of hexachlorobenzene and *p,p'*-DDE and smaller concentrations of *p,p'*-TDE and dieldrin were estimated by most analysts. Lindane (γ -BHC), *p,p'*-DDT, and heptachlor epoxide were reported by a few laboratories. The results are given in Table 7.

Fourteen laboratories reported on the sample, three giving several estimates of the PCB content by various methods of calculation or analysis. The reference solutions used included 50%, 54%, and 60% chlorinated types. The overall range of all results reported was 240-525 mg/kg in the original tissue, but for eight results (using a 60% chlorinated reference solution) the range was 279-462 mg/kg, with a mean of 374 mg/kg and a

TABLE 5.—Comparison of analytical efficiencies for determination of organochlorine residues in 1967-68 and 1969-71 samples

COMPOUND	COEFFICIENT OF VARIATION AND NUMBER OF RESULTS ()		
	1967-68 (1)		1969-71
	STANDARDS IN HEXANE	CHICKEN EGG	SPIKED CORN OIL
Heptachlor epoxide	± 11.0% (15)	—	±16.8% (18)
Dieldrin	± 7.4% (17)	±28% (15)	±15.9% (18)
<i>p,p'</i> -DDE	± 9.9% (16)	±32% (15)	±14.4% (19)
<i>p,p'</i> -TDE	± 7.5% (17)	±51% (11)	± 9.8% (17)
<i>p,p'</i> -DDT	± 7.0% (17)	±31% (15)	±16.1% (19)

TABLE 6.—Analyses of test sample no. 2—PCB solution

LABORATORY	REFERENCE	PCB CONCENTRATIONS IN MG/LITER
Canada		
FRB	Aroclor 1260	9.0
ORF	Aroclor 1260	11.4
Denmark	Clophen A.-60	9.3
Euratom	Clophen A.-50	8.4
Finland		
Agric.	Clophen A.-60	10.0
Vet. Med.	Clophen A.-60	10.0
Netherlands		
CIVO	Clophen A.-60	10.4
RIV	Phenoclor DP. 6	12.6
Norway	Clophen A.-50	8.0
Spain	Clophen A.-50	8.0
United Kingdom		
FFL (two GLC columns)	Aroclor 1260	11.0
	Aroclor 1260	9.6
	Aroclor 1260	10.4
LGC	DDE	8.9
United States		
EPA	Aroclor 1260	9.1
USDI	Aroclor 1260	9.3
True Value	Clophen A.-60	9.8

coefficient of variation of $\pm 15.8\%$. This agreement among the analysts would seem to be very satisfactory, in view of the many differences in extraction, cleanup, and separation techniques and the different methods of estimating the PCB concentrations. The coefficient of variation among these results is within the range of values found for the pesticides in the corn oil sample (No. 1) which required less processing.

Of the other residues found, 12 results were reported for *p,p'*-DDE, from 4.5 - 19.5 mg/kg. Some laboratories apparently did not correct for PCB interference with this residue but those which did found concentrations of *p,p'*-DDE from 4.5 to 11.0 mg/kg. Eight values for dieldrin were between 0.9 and 2.2 mg/kg but a ninth

value was less than 0.1 mg/kg. Failure to obtain a complete separation of PCB's from *p,p'*-TDE and *p,p'*-DDT would have resulted in significant errors for these residues in this particular sample; however, only two values of *p,p'*-DDT were recorded, and both were very low. The only other significant residue found was hexachlorobenzene (HCB), for which six values ranging from 22.3 to 48.7 mg/kg were reported, but not all laboratories recognized or were able to determine this substance.

This sample was not particularly easy to analyze, but the agreement obtained was good for the major contaminant, and much better than for the residues in the sprat homogenate used in the previous study. The higher concentrations in the cormorant sample probably contributed to the better agreement among analysts.

Sample No. 4 (mercury in fish)

Analyses of the three samples of fish tissue for total mercury and/or methylmercury were made by nine laboratories. Values for total mercury from four of them were in good agreement, and the eight sets of data on methylmercury were in reasonably good agreement (Table 8).

The mean value for the mercury content of Sample A was 0.152 mg/kg as methylmercury and 0.165 mg/kg as total mercury. Sample B was prepared from an identical aliquot of Sample A, with the addition of a fixed amount of an aqueous solution of methylmercury dicyandiamide. The mean values calculated for this spiked solution were equivalent to 0.36 mg/kg as methylmercury and 0.39 mg/kg as total mercury, the true value being given as 0.35 mg/kg. For Sample C, the mean values were 1.89 mg/kg as methylmercury and 2.12 mg/kg as total mercury. The coefficient of variation among the eight laboratories reporting methylmercury was $\pm 20\%$ for the spike, and $\pm 28\%$ for Sample C (high mercury). The methylmercury estimated in Sample C represented about 90% of the total mercury found.

The methods used for methylmercury analysis varied among the laboratories, but all used either a hydrochloric acid, hydrobromic acid, or cupric bromide reagent to liberate the methylmercury from the fish tissue, followed by extraction into an organic solvent. Some analysts used a cleanup stage with cysteine acetate and re-extraction into a further solvent, but others did not find this step necessary. The concentrations of methylmercury in the extracts were determined by electron-capture gas chromatography in all laboratories. The Euratom Laboratory determined total mercury using neutron activation analysis, but all other laboratories used flameless atomic absorption, with different types of digestion mixture.

TABLE 7.—Analyses of test sample no. 3—cormorant

LABORATORY	CONCENTRATIONS IN MG/KG								PCB REFERENCE
	HCB	γ-BHC	HEPTACHLOR EPOXIDE	DIELDRIN	p,p'-DDE	p,p'-TDE	p,p'-DDT	PCB's	
Canada									
FRB	34.0	0.50	0.13	1.13	14.3	1.56		402	Aroclor 1254
ORF	48.7			2.02	18.4	2.92		462	Aroclor 1260
Denmark					14			374	Clophen A.-60
Euratom		3.3		<0.1	13.3	2.4		432	Clophen A.-60
Finland									
Vet. Med.			(¹)	(¹)	(¹)	(¹)		279	Clophen A.-60
Agric., (two methods)			(¹)		± 6.5	4.5		525	Clophen A.-50 & A.-60
			(¹)	2.2	± 4.5	4.1		450	Clophen A.-50 & A.-60
Germany									
Munich, (three methods)								395, 378, 360	Clophen T.-64
Netherlands									
CIVO	44				19.5			400	Clophen A.-60
Norway								273	Clophen A.-50
Spain	(¹)	0.3		1.4	± 9.3	2.7	0.3	320	Clophen A.-60
United Kingdom									
FFL	22.3			0.97	± 8.2	1.5	0.18	283	Aroclor 1254
LGC, (two methods)	23	0.08		0.9	± 9.7	1.4		345	Clophen A.-60
	28	0.08		1.1	± 10.0	1.6		340	DDE or Dieldrin
United States									
EPA				1.3	± 11	2.2		470	Aroclor 1254
USD1								240	Aroclor 1254

¹ Detected, but value not estimated.² Corrected for PCB interference.

TABLE 8.—Analyses of test sample no. 4—pike homogenate

LABORATORY	CONCENTRATIONS IN MG. KG							
	METHYL MERCURY				TOTAL MERCURY			
	A	B	B-A	C	A	B	B-A	C
Canada								
ORF	0.18	0.64	0.46	1.27	0.20	0.56	0.36	2.11
Denmark	0.14	0.46	0.32	1.97				
Euratom					0.15	0.70	0.55	2.0
Finland								
Vet. Med.	0.14	0.50	0.36	2.35				
Germany								
Munich	0.14							
Norway	0.13	0.59	0.46	2.60				
United Kingdom								
FFL	0.18	0.56	0.38	2.0	0.13	0.42	0.29	2.18
LGC, (two methods)	0.13	0.40	0.27	1.4	0.18	0.54	0.36	2.2
	0.14	0.46	0.32	1.4				
United States								
USD1	0.18	0.47	0.29	2.60				
Mean	0.152	0.511	0.357	1.89	0.165	0.555	0.39	2.12
True Value	0.35				0.35			

CONCLUSIONS (BASED ON TEST SAMPLES)

The results of this section of the program represent a considerable improvement over those of the previous studies. Analysts using a variety of methods were able to achieve reasonably good agreement on the amounts of both organochlorine pesticide and PCB residues in wildlife samples requiring full processing, and the techniques for separation of PCB's from most organochlorine pesticides seemed to be adequate for this purpose. From the details available, there was no evidence that any one technique was consistently better than another. Where two methods were compared in the analysis of the corn oil sample, as reported by two laboratories, the within-laboratory agreement was excellent. Although the estimation of PCB residues is clearly dependent on the reference formulation and the method of calculation (e.g., using the summation of peak heights or areas of one or more peaks), the general agreement on total PCB levels was nevertheless satisfactory, bearing in mind that the composition of the mixture of PCB isomers in wildlife is rarely typical of commercial mixtures. If international monitoring programs for organochlorines and mercury are established, it is unlikely that any one method of analysis would be

acceptable to all laboratories, but this study has shown that it is possible to achieve good agreement among laboratories using different methods in the hands of experienced analysts.

Organochlorine Residues in Wildlife Samples

SAMPLING PROCEDURES (WILDLIFE SAMPLES)

This part of the study was intended to be more extensive than that in the previous 2-year program (1) and was concentrated on the aquatic environment, including where possible both ostensibly uncontaminated and recognizably contaminated areas, to determine whether significant differences in residue levels could be detected. The numbers of individuals selected were usually sufficient to confirm a two-fold difference, according to the results of the previous study. The soft tissue of fish and shellfish and the contents of the eggs of birds were selected for analysis. In addition, eggshell thickness indices were to be calculated for the eggs and, where possible, breeding success in an attempt to determine any biological effects of the residues on the bird population.

The freshwater fish species selected were the pike (*Esox lucius*) or if not available, the roach (*Rutilus rutilus*), or eel (*Anguilla vulgaris*). The marine species were the mussel (*Mytilus edulis*), herring (*Clupea harengus*), or the sprat (*Clupea sprattus*) where the herring was not available. The bird species, which were all piscivorous, were to be chosen from the genera *Ardea*, *Pelecanus*, *Podiceps*, *Uria*, *Alca*, *Sterna*, *Phalacrocorax*, and *Somateria*. The numbers to be taken and the sampling times were specified as indicated below.

The detailed procedures for sample collection and preparation were specified as follows:

Pike, roach, or eel—15 specimens to be collected before spawning from each of both uncontaminated and contaminated sites, the individuals to be of uniform size, and if possible of the same sex. Five fish to be assigned randomly to each of three pools, and each pool to be homogenized from whole fish or from 10-g aliquot of lateral muscle taken at the midsection, avoiding depot fat. The six pools for each species were to be analyzed for organochlorine residues, and if possible mercury. Fat contents of tissues were to be determined if possible.

Herring or Sprat—procedures were the same as for freshwater fish, except that eight fish were to be assigned to each pool.

Mussel—to be collected from at least one contaminated site and one uncontaminated site in estuaries or at the mouths of large rivers. Thirty large specimens to be collected before spawning and divided randomly into 3 pools of 10. The soft parts were to be homogenized in

each pool and analyzed for organochlorines and, if possible, mercury residues. Each pool was to be analyzed in duplicate.

Eggs from fish-feeding colonial birds—the species selected were to be sampled in areas where organochlorines were believed to be contaminating the water and in areas where they were not. The nests were to be counted in each colony. At each site, 10 eggs were to be taken from separate nests as soon after laying as possible, and each egg was to be analyzed separately for organochlorines and, where possible, mercury.

The eggshell index was to be measured from the formula:

$$\text{Eggshell Index} = \frac{\text{Weight in mg}}{\text{Length in mm} \times \text{breadth in mm}}$$

The measurements were to be made to 0.01 g or 0.01 mm, and the weight of the evacuated shell determined after drying for 1 month in air.

The breeding success was to be studied by recording the number of breeding pairs in the colony, and the number of young reared during the season, each number being determined on 1 day during egg laying (number of pairs) or before dispersal (number of large young).

RESULTS (WILDLIFE SAMPLES)

Ten countries were involved in sampling at least one of the selected species: all 10 examined the mussel, 8 the herring (or sprat), and 6 the pike. Several countries reported difficulties in conforming exactly to the specified procedure, especially in respect to sampling times and numbers of individuals required, and it was not always possible to sample in both contaminated and uncontaminated areas. Although the mussel, herring, and pike were well covered by a number of countries, enabling some degree of comparison to be made between countries, most species of birds were examined by only one country, the eider (*Somateria mollissima*) being sampled by three, and the heron (*Ardea cinerea*) by two countries. Three different species of tern (the Royal tern—*Thalasseus maximus*, common tern—*Sterna hirundo*, and Sandwich tern—*Sterna sandvicensis* and of the other selected genera, the eastern brown pelican (*Pelecanus occidentalis carolinensis*), were examined by one country.

Summaries of the results reported in this study are given in Tables 9-15. In some instances, no clear distinction was made between contaminated and uncontaminated areas, and some contamination was of minor importance. Pollution by sewage from towns discharging to estuaries or the sea was sometimes assumed to be a source of organochlorine pollution, and in a few instances samples could not be obtained from appropriately polluted areas.

Because some countries could not obtain sufficient individuals for analysis as required by the defined procedure, statistical comparison of the data was not justified, although two-fold or larger differences can be regarded as significant where at least 10 individuals were analyzed. Where residues were not detected, the limit of detection was stated. Moreover, the analytical results were incomplete since some residues were not analyzed for by all laboratories. Both marine and freshwater species usually contained dieldrin, DDE, TDE, and DDT, as well as PCB's; however, some laboratories did not indicate whether analyses for all these residues had been attempted. Mercury was not determined by all

laboratories since the necessary procedure was not available to all those engaged in the examination of organochlorines. A few laboratories reported on residues of HCB, α -BHC, and γ -BHC, although evidence of confirmation was not necessarily given.

A discussion of the results obtained for the various species is given below:

Mussel (examined by 10 countries—Table 9)

Most investigators analyzed pools of 10 mussels, as required by the program, but in a few cases the pools

TABLE 9.—Analyses of wildlife samples—mussels

LOCATION	NUMBER OF MUSSELS IN SAMPLE	MEAN VALUES IN MG/KG						POLLUTION CATEGORY
		DIELDRIN	DDE	TDE	p,p'-DDT	PCB's	Hg	
Canada								
Miramichi Bay	30	0.02	0.02	0.01	0.01	0.14	0.02	Polluted
Denmark								
Vejle	2	0.005	0.005	0.004	0.002	0.06		Slightly polluted
Esbjerg	3	0.011	0.016	0.004	0.004	0.28		Slightly polluted
Finland								
Tvärminne	60	<0.005	<0.005	<0.005	0.008	0.033	0.04	Unpolluted
Södarskär	20	<0.005	<0.005	<0.005	0.020	0.027	0.03	Unpolluted
Netherlands								
1969 Vlieland	5	0.016	0.016			1.7	0.14	Slightly polluted
Noordpolderzijl	5	0.007	0.006			0.87	0.13	Slightly polluted
1970 Noordpolderzijl	10	0.010	0.013			0.28	0.08	Slightly polluted
Ijmuiden	35	0.016	0.020			0.45	0.21	Slightly polluted
Scheveningen	27	0.014	0.018			0.38	0.31	Slightly polluted
Hoek van Holland	31	0.017	0.027			0.21	0.22	Slightly polluted
Grevelingen W	23	0.010	0.016			0.47	0.11	Slightly polluted
Grevelingen O	10	0.007	0.023			0.52	0.14	Slightly polluted
Vlissingen	25	0.023	0.022			0.48	0.14	Slightly polluted
Cadzand	28	0.008	0.015			0.33	0.21	Slightly polluted
Norway								
Hurumlandet	15	<0.005	<0.005	<0.005	<0.005	<0.005		Unpolluted
Portugal								
Aveiro	60	<0.001	0.002	0.001	0.004	0.03		Unpolluted
Cascais	36	0.005	0.007	0.012	0.029	0.17		Polluted
Spain								
Blances	30	0.002	0.035	0.011	0.012	0.58		Polluted
Barcelona	30	<0.001	0.054	0.034	0.010	1.05		Polluted
Ampolla	30	<0.001	0.137	0.061	0.005	0.77		Polluted
Castellon	30	0.025	0.001	0.014	0.010	0.61		Polluted
Vigo	30	<0.001	0.005	0.006	0.006	0.10		Unpolluted
Santander	30	0.001	0.002	0.025	0.006	0.67		Unpolluted
Sweden								
Fiskebäckskil	90		0.003	0.002	0.004	0.041	0.15	Unpolluted
Askö	90		0.008	0.003	0.005	0.017	0.03	Polluted
Gräddö	90		0.008	0.002	0.005	0.017	0.08	Polluted
United Kingdom								
Montrose	30	0.010	0.006	0.023	0.019	<0.1		Unpolluted
Lochgailhead	30	0.013	0.014	0.009	0.013	<0.1		Unpolluted
Ythan Estuary	30	0.003	0.011	0.006	0.006	0.1	0.23	Unpolluted
Helensburgh (Clyde)	30	0.059	0.035	0.037	0.046	0.62	0.07	Polluted
Kilcreggan (Clyde)	30	0.074	0.034	0.031	0.032	0.48	0.04	Polluted
Holy Loch (Clyde)	30	0.026	0.016	0.012	0.017	0.44	0.46	Polluted
United States								
New York	30	0.032	0.025	¹ 0.069	¹ 0.073	Present		Polluted
Cape Split	30	<0.010	<0.010	<0.010	<0.010	Present		Unpolluted

NOTE: Where residues were not detected, the limit of detection was stated.

¹ PCB's not separated.

were larger or smaller, or the means of analyses of individual specimens were reported.

Except for PCB levels in the order of 1 mg/kg and mercury levels approaching 0.5 mg/kg, none of the residues reported were particularly high. For all organo-chlorine residues and mercury, there was a considerable overlap in the ranges of concentrations found in the areas classified as unpolluted or polluted. The lowest concentrations reported in the two classifications were very similar, while at the upper ends of the ranges the amounts found in the polluted areas were only about twice those found in the ostensibly unpolluted areas. This failure to demonstrate a clear distinction due to pollution may be the result of an inability to identify pollution from the outward circumstances, or perhaps to the capability of mussels to adjust their residue levels rapidly with changing environmental concentrations. A sustained high level of contamination in the local environment should nevertheless be reflected in relatively high concentrations in mussel tissue.

The highest dieldrin concentrations were reported in mussels from the Clyde (United Kingdom), an estuary which receives discharges from factories using the chemical for moth-proofing. DDT group residues were highest in samples from the United Kingdom and the Mediterranean coast of Spain, while PCB residues were highest in the Netherlands, Spain, and the Clyde estuary (United Kingdom).

Herring (examined by eight countries—Table 10)

Although this species (or the closely-related sprat and sardine) was sampled in off-shore waters which might be expected to be less contaminated because of the greater dilution factor than the littoral zone in which mussels were sampled, the concentrations of the various residues found were often higher than those in mussels. Again, there was a considerable overlap between the ranges of values reported for uncontaminated and contaminated areas. Some PCB and DDT group residues were noticeably higher in herring than in mussels from

TABLE 10.—Analyses of wildlife samples—herring

LOCATION	NUMBER OF FISH IN SAMPLE	MEAN WEIGHT (G)	MEAN RESIDUES IN MG/KG						POLLUTION CATEGORY
			DIELDRIN	DDE	TDE	p,p'-DDT	PCB's	Hg	
Canada									
Chedabucto Bay	10	222	<0.02	0.24	0.04	0.15	0.54	0.05	Unpolluted
	10	59	<0.02	0.09	0.02	0.08	0.32	<0.01	Unpolluted
Bay of Fundy	10	16	<0.02	0.06	0.01	0.05	0.34	<0.01	Polluted
Denmark									
Esbjerg	2		0.003	0.02	0.015	0.022	0.10		Unpolluted
Grenå	2		0.015	0.21	0.115	0.28	0.46		Unpolluted
Neksø	2		0.020	0.57	0.18	0.52	0.42		Polluted
Finland									
Haapasaari		60	<0.02	1.10	0.40	0.40	0.40	0.12	Slightly polluted
		30	<0.02	0.25	0.20	0.10	0.19	0.14	Slightly polluted
Kotka		23	<0.02	0.12	0.10	0.08	0.21	0.05	Slightly polluted
Netherlands ¹									
Wadden Sea	16		0.032	0.042			0.78	0.19	Slightly polluted
Portugal ²									
Arrifana	24	47	0.007	0.029	0.007	0.020	0.12		Unpolluted
Algarve	24	36	0.005	0.052	0.052	0.017	0.029		Unpolluted
Spain ²									
Palamos	24	36	0.043	0.51	<0.001	<0.005	3.2		Polluted
Barcelona	24	44	0.049	0.46	0.033	<0.005	3.1		Polluted
Castellon	16	30	<0.001	0.12	<0.001	<0.005	0.62		Polluted
Vigo	16	60	0.007	0.08	0.010	<0.006	0.32		Unpolluted
Santander	24	85	<0.001	0.001	<0.001	<0.005	0.25		Unpolluted
Sweden									
1969 Bornholm Basin	60			0.8	0.4	1.0	1.1	0.06	Polluted
Gulf of Bothnia	25			0.18	0.06	0.17	0.36	0.11	Unpolluted
1970 Bornholm Basin	103			0.6	0.4	0.6	0.47	0.04	Polluted
Gulf of Bothnia	45			0.30	0.07	0.22	0.67		Unpolluted
United Kingdom									
Eyemouth	10	190	0.017	0.044	0.016	0.031	0.29	0.02	Unpolluted
Clyde	24	190	0.109	0.099	0.062	0.070	0.79	0.07	Polluted
S. Ireland								0.12	Unpolluted
Lowestoft								0.09	Unpolluted

NOTE: Where residues were not detected, the limit of detection was stated.

¹ Includes sprat.

² Sardines.

the same area, especially in the samples from Sweden and the United Kingdom (Clyde). This was only partly due to the difference in lipid content since Swedish samples also showed a marked difference even when calculated on a lipid basis.

The highest dieldrin concentrations were reported from the United Kingdom, the highest DDT values from the Baltic (Finland and Sweden), and the highest PCB values from Spain. None of the mercury concentrations exceeded 0.2 mg/kg.

Pike (examined by six countries—Table 11)
Once again the overlap between the ranges of residue concentrations reported from uncontaminated and contaminated areas was considerable. The residue levels of organochlorines in pike from all areas were lower than those in herring, but mercury concentrations were significantly higher, even in unpolluted areas. High concentrations of mercury have been found in pike from unpolluted areas in several countries and are generally accepted as of natural origin.

Dieldrin levels in pike were generally low; the highest DDE values were reported by Spain and the highest PCB values from the Netherlands. Finland reported the highest mercury concentrations, one mean value being almost 4 mg/kg.

Eel (examined by two countries—Table 12)
The few results reported gave no clear indication of any difference between unpolluted and polluted waters, but the concentrations of organochlorines in eels, probably because of the high lipid content of eel muscle, were much higher than those reported for pike. This species seems worthy of further study to determine why differences in pollution status were apparently not reflected in the residue levels in the samples examined.

Heron eggs (examined by two countries—Table 13)
In the eggs of all the species of birds examined dieldrin, TDE, and DDT residues were generally much smaller than those of DDE and PCB's. For the heron eggs, there was a marked difference between the contamination levels in unpolluted and polluted areas, the maximum values of the concentrations of different contaminants in the polluted areas being up to two orders greater than in the clean areas. The levels were also much greater than in the freshwater fish examined, although none of the fish and egg samples were from the same area in any country. From the British data, the eggshell thickness index of the heavily contaminated eggs was about 10% less than that of the uncontaminated eggs.

Eider eggs (examined by three countries—Table 14)
None of the samples analyzed were from heavily contaminated areas, although the PCB concentrations

TABLE 11.—Analyses of wildlife samples—pike

LOCATION	NUMBER OF FISH IN SAMPLE	MEAN RESIDUES IN MG/KG						POLLUTION CATEGORY
		DIELDRIN	DDE	TDE	p,p'-DDT	PCB's	Hg	
Finland								
Kivijärvi	5	<0.005	0.006	<0.005	0.012	0.008	0.27	Unpolluted
Kotka	5	<0.005	0.009	<0.005	<0.005	0.033	2.12	Slightly polluted
Ahvenkoski	5	<0.005	0.017	<0.005	<0.005	0.023	2.95	Polluted
Tammijärvi	5	<0.005	0.005	<0.005	<0.005	0.030	3.85	Polluted
Netherlands								
Grouw	5	<0.006	0.054			0.47	0.25	Slightly polluted
Westeinder	5	0.013	0.144			0.60	0.36	Slightly polluted
Dobersdorfer See ¹	7	<0.013	0.115			0.71	0.24	Slightly polluted
Warder See ¹	3	<0.006	0.170			0.95	0.42	Slightly polluted
Norway								
Øyeren	10	<0.005	0.005	<0.005	<0.005	<0.005		Polluted
Langen	10	<0.005	0.005	<0.005	<0.005	<0.005		Unpolluted
Spain								
Garcia Sola	2	0.003	0.46	0.002	0.009	0.13		Polluted
Orellana	4	0.003	0.48	0.044	<0.006	0.12		Polluted
Sweden								
1970 Bolmen	10		0.018	0.007	0.014	0.030	0.45	Slightly polluted
Storvindlen	10		0.006	<0.001	0.003	0.019	0.28	Unpolluted
1971 Bolmen	10		0.022	<0.001	0.010	0.087	0.36	Slightly polluted
Storvindlen	10		0.006	<0.001	0.010	0.040	0.29	Unpolluted
United Kingdom								
Abberton Res.	13						0.68	Unpolluted
River Chelmer	15						0.65	Polluted
Loch Leven	10	0.004	0.030	0.016	0.009	<0.01	0.08	Slightly polluted

NOTE: Where residues were not detected, the limit of detection was stated.
¹ Samples from West Germany.

ranged from 0.27 to 9.3 mg/kg. The mean eggshell thickness index of 2.16 in the Finnish sample was significantly different (at the 5% level) from that of the Norwegian sample, 2.28, and the mean PCB value was about seven times greater. The eggshell thickness index was unfortunately not given for the Danish eggs, which contained the most PCB's.

Tern and pelican eggs (examined by three countries—Table 15)

The eggs of three species of tern and one species of pelican, although not strictly comparable, are discussed collectively because of the information given, both on residue levels and eggshell thickness indices, together with some observations on the breeding success. Two

TABLE 12.—Analyses of wildlife samples—eel

LOCATION	NUMBER OF EELS IN SAMPLE	MEAN RESIDUES IN MG/KG						POLLUTION CATEGORY
		DIELDRIN	DDE	TDE	p,p'-DDT	PCB's	Hg	
Canada								
Chamcook Lake	3	<0.02	0.55	<0.01	0.21	0.63	0.35	Unpolluted
St. John River	10	<0.02	0.57	0.27	0.32	0.75	0.72	Polluted
United Kingdom								
River Eden	15	0.52	0.83	0.51	0.67	<0.1	0.31	Polluted
Leader Water	15	0.77	1.07	0.31	0.71	<0.1	0.24	Unpolluted
Oxnam Water	15	0.70	0.85	0.50	0.80	<0.1		Unpolluted
Leet Water	15	0.95	0.59	0.42	0.36	<0.1	0.22	Polluted

NOTE: Where residues were not detected, the limit of detection was stated.

TABLE 13.—Analyses of wildlife samples—heron eggs

LOCATION	NUMBER OF EGGS IN SAMPLE	MEAN WEIGHT (g)	MEAN EGGSHELL INDEX	MEAN RESIDUES IN MG/KG				POLLUTION CATEGORY
				HCB	DIELDRIN	DDE	PCB's	
Netherlands								
Amsterdam	9			1.2	1.9	7.4	74	Polluted
Edam	4			1.0	1.1	1.7	34	Slightly polluted
Eindhoven	8			0.36	0.5	2.8	33	Slightly polluted
Stamper Toren	6			0.64	1.5	5.9	34	Slightly polluted
United Kingdom								
Denver	13	52.2	1.49		1.12	6.65	7.3	Very polluted
Troy	8	54.0	1.56		0.80	3.17	3.25	Polluted
Shieldaig	4	43.1	1.62		<0.03	0.85	<0.5	Unpolluted
Druidibeg	4	56.3			0.05	1.35	<0.75	Unpolluted
Carrowmore	7	42.3	1.65		0.07	0.41	<0.5	Unpolluted

NOTE: Where residues were not detected, the limit of detection was stated.

TABLE 14.—Analyses of wildlife samples—common eider eggs

LOCATION	NUMBER OF EGGS IN SAMPLE	MEAN WEIGHT (g)	MEAN EGGSHELL INDEX	MEAN RESIDUES IN MG/KG						POLLUTION CATEGORY
				DIELDRIN	DDE	TDE	DDT	PCB's	Hg	
Denmark										
1970 Hov Røn	10				0.56			9.3		Slightly polluted
1971 Hov Røn	10				0.35			4.4		
Finland										
Söderskär	14	101.8	2.16	0.070	1.05	0.08	<0.01	1.8	0.33	Unpolluted
Norway										
Grindøy	12	114.0	2.28	<0.01	0.40	<0.01	<0.01	0.27		Unpolluted

NOTE: Where residues were not detected, the limit of detection was stated.

TABLE 15.—Analyses of wildlife samples—tern and pelican eggs

LOCATION	NUMBER OF EGGS IN SAMPLE	SPECIES	MEAN EGGSHELL INDEX	MEAN RESIDUES IN MG/KG (FRESH WEIGHT)							POLLUTION CATEGORY
				HCB	DIELDRIN	DDE	TDE	DDT	PCB's	Hg	
Canada											
Bathurst, N.B.	10	Common tern	—	0.028	0.041	0.91	0.016	0.073	2.36	0.124	Slightly polluted
Hamilton, Ont.	10	Common tern	0.831	2.31	0.76	23.1	0.73	0.49	149	0.87	Polluted
Netherlands											
Wadden See	10	Sandwich tern	1.13	0.103	0.082	0.51			8.2	1.7	
United States											
South Carolina	10	Royal tern	1.47		0.2	2.8	0.04	<0.1	4.5	0.8	Polluted
		Pelican	2.28		0.7	3.3	0.7	0.6	4.5	0.5	Polluted

NOTE: Where residues were not detected, the limit of detection was stated.

populations of common terns examined in Canada, from an uncontaminated and a highly contaminated area, showed large differences in all the residues determined, two orders of magnitude in the case of HCB and PCB's. Although the eggshell thickness index of the eggs from the uncontaminated colony (Bathurst) was not determined, the heavily contaminated eggs (Hamilton) showed a decrease of 10% as compared with the index of all Canadian eggs measured before 1935, and the shells were mechanically weaker. The reproductive success of the Hamilton colony, as measured by percentage of fledglings from eggs laid or fledged young per adult pair, was only 16-23% of that of the Bathurst colony, the decrease apparently unlikely to have resulted from either the lower density of the colony or disturbance by observers.

The high concentrations of some residues found, 2.3 mg/kg for HCB, 23.1 mg/kg for DDE, and 149 mg/kg for PCB's, were greater than for any other species reported in the study. These values were given on a wet-weight basis, but when expressed on a dry-weight basis to allow for differences in condition, the marked contrast in residue concentrations between the two sites remained.

The population of Sandwich terns studied in the Netherlands was known to have been heavily contaminated by cyclodiene insecticides in earlier years, but telodrin was absent from the 1970 egg sample and dieldrin and endrin were much lower than in 1965. The PCB and mercury contents were higher than in the uncontaminated Canadian sample of common tern eggs, but much lower than in the contaminated Canadian eggs, and the eggshell thickness index of the Netherlands sample was estimated to have been only about 5% less than in 1950.

In the United States, a population of Royal terns was studied, this stock having shown no decrease since 1947. The mean residue levels (except for DDT) were higher than those in the Bathurst colony of common terns from

Canada, and not greatly different from those in the Sandwich tern eggs from the Netherlands. By comparison, the eggs of brown pelicans taken in the same area of the United States had residue concentrations similar to those in the Royal terns, yet the pelican colony has declined by over 80% in less than a decade and is now considered in danger of extinction. The eggshell thickness index of the pelican eggs has declined by 14% since 1947.

CONCLUSIONS (BASED ON WILDLIFE SAMPLES)

The results from the wildlife sampling program have emphasized the difficulties involved in selecting appropriate species for international monitoring, in obtaining adequate samples of individuals for analysis, in identifying areas of high contamination, and in relating the residue concentrations found to any measurable biological effect. Despite the detailed manner in which the sampling requirements of the biological program were described, some investigators did not follow these closely.

Perhaps the most surprising finding of the biological sampling program was that the differences in residue concentrations between samples of the same species of fish and shellfish from selected unpolluted and polluted areas were often small and, in respect to the combined samples from all countries, not significant. Thus, what may be considered a polluted area in one country could be classified as unpolluted in another, rendering the interpretation of chemical data from a global monitoring program very difficult. Even in the apparently unpolluted areas, where contamination is largely the result of atmospheric fallout or washout or oceanic circulation, the range of values found was considerable for most species examined.

Thus, for mussels, PCB concentrations in areas apparently free of local pollution ranged from below 0.01 to 0.67 mg/kg, and in herring the DDE concentrations

ranged from less than 0.01 to 0.30 mg/kg. Assuming the level representing background contamination from globally distributed material in the period 1969-71 to be below 0.01 mg/kg, a number of samples taken from areas selected as free of local pollution must nevertheless have had contamination significantly above the baseline level. A much greater number of widely distributed samples would be required to estimate the baseline level of contamination with a satisfactory degree of precision for any one species.

The information obtained from eggs of piscivorous birds was more limited than that from the fish and shellfish, as few species were sampled by more than one country. Yet the differences between the uncontaminated and the contaminated areas, as demonstrated by those species sampled by more than one country, were much larger than was found for fish. Although it was hoped that measurements of eggshell thickness index and reproductive success would help to establish the extent of the effects of such differences, several investigators did not find it possible to complete such measurements. The information supplied does, however, confirm that what may be regarded as a harmful residue level in one species is apparently harmless in another. A 14% decrease in eggshell thickness index in pelican eggs seems to be a significant ecological factor, as does a 10% decrease in common tern eggs, but the latter was produced by a much higher organochlorine level than occurred in the pelican eggs (although the correlation between organochlorines and eggshell thickness index decrease is only presumptive at the present time).

From the results of the chemical exchange sample program, it is assumed that analytical accuracy is such that the major differences in residue levels found in the wildlife specimens are not due to variations between analysts or analytical techniques. The differences between contamination levels in the various populations were often very much larger than those now expected among the group of analysts who participated in the program. Before any international monitoring system is established to assess the level of contamination of the environment and the changes which may follow government action on pollution, closer attention must be paid to the choice of appropriate indicator species and to obtaining adequate numbers of individuals for analysis in a sufficient number of sampling sites. There are very few globally distributed species and probably none ideally suitable for monitoring, but species representing sufficiently large areas would provide adequate information for indicating general trends.

The problem of assessing the ecological significance of even the high concentrations found in some populations nevertheless remains a major issue. Unless some measure-

ments of appropriate biological parameters can be made in conjunction with the chemical analyses, in both experimental laboratory and field studies, an effective ecological evaluation of chemical data from monitoring programs will be impossible. At the present time, the only practical field measurements which might be correlated with levels of contamination are those for egg-shell thickness and reproductive success in birds; no comparable information is obtainable for fish populations, yet the residue levels in fish may be important for piscivorous birds, even though they may appear harmless to the fish themselves. Judgment of the significance of residues in fish can therefore, in some instances, be based on the wider ecological implication of their ultimate effect on the predator species.

Further Programs

This program has shown that, apart from an improvement in the ability of analysts to agree on both the identity and quantity of the major organochlorine residues and an encouraging measure of agreement on mercury residues, the biological sampling and assessment of ecological changes falls far short of that necessary for an effective monitoring program. At the Third O.E.C.D. Technical Conference in Berlin in January 1972, it was emphasized that any further study should be given official support by member governments to ensure that the program would be effectively fulfilled. The O.E.C.D. Sector Group on the Unintended Occurrence of Chemicals in the Environment, at a meeting in Paris in March 1972, endorsed this view and approved a further study with specific objectives of direct interest to member governments. Briefly, the study involves the sampling of populations of herring (or sardine) in the marine environment, perch (or roach) in the freshwater environment, and nestling starlings (*Sturnus vulgaris*) in the terrestrial environment. Twenty-five individuals of the same age, and as far as possible of uniform sex and size, are to be analyzed individually for organochlorines and mercury. In addition, 10 pike of varying size but of one sex are to be taken from each appropriate location for mercury analysis, using the regression of mercury concentration on weight to calculate the mercury content of a 1-kg weight fish. Fish samples are to be taken prior to spawning, and the program will be repeated annually in the same locations for a further 2 years.

From this study, an attempt will be made to determine whether a statistically significant decrease in the environmental levels of DDT, PCB's, or mercury can be found in any of the selected locations, following the action of a number of governments and industries in reducing the usage or discharge to the environment of these chemicals at the present time. It is not anti-ci-

pated that all areas would show a decrease in the period under study, but it is hoped that such a change may be detected in at least some areas. It is clearly desirable that, as far as possible, action by governments and industries to reduce environmental contamination can be shown to be followed in due course by declines in the environmental levels of the substances controlled, but the rates of such declines are at present purely speculative.

See Appendix for chemical names of compounds discussed in this paper.

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Pesticide Residues in Natural Fish Populations of the Smoky Hill River of Western Kansas—1967-69¹

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ABSTRACT

Fish populations from five locations in an area of low pesticide use are described and their pesticide residue levels are given for the 3-year period, 1967-69. The study was carried out in a dry-land farming area with developing irrigation on the Smoky Hill River of west central Kansas. Fish samples were collected by electro-shocking at five stations along an 80-km section of the river. A total of 393 samples of the commonly occurring species (whole body, flesh, gonads) were analyzed for organochlorine residues.

During the 3 years, 24 species of fishes were collected; 15 of these species were common in the study area. The number of species, number of individuals collected, and species diversity are presented for each station with respect to time. No endrin, aldrin, or heptachlor residues were detected in any sample. Heptachlor epoxide was found in trace amounts in three samples. Dieldrin was present in 15% of the samples, usually in amounts less than 0.01 ppm. DDT and its metabolites were detected in 75% of the samples, usually at only several hundredths of a part per million. The fish populations collected were considered "clean" from pesticides when compared with fishes from other surveys. These data form a baseline for future comparison as the agriculture changes in this area.

Introduction

Pollution of the environment and its potential effects on the health of man, animals, fish, wildlife, and the ecosystem in general have become subjects of concern. Insecticides have greatly benefited man by reducing disease and increasing food production; they also have been a major source of pollution (13), particularly to fish (6, 7).

Insecticides have been used extensively, and fairly high residue levels have been detected in fish from widely scattered areas (4,5). A unique opportunity to study the accumulation of pesticide residues and their effect on natural fish populations was provided as the Cedar Bluff Irrigation District, formerly a dry-land farming area with little insecticide usage, developed and as crop production and insecticide usage intensified (3,10).

This study describes the natural fish populations in a segment of the Smoky Hill River in the vicinity of Cedar Bluff Irrigation District, and presents data on pesticide residues in the common species of fishes in the area during the 3-year period, 1967-69.

Methods

THE STUDY AREA

Samples were collected from the Smoky Hill River in Trego and Ellis Counties in west central Kansas (Fig. 1). Typical of the Great Plains Region, the topography is flat to rolling with occasional breaks and canyons. The climate is continental with average annual precipitation of about 54 cm.

The Smoky Hill River originates in eastern Colorado and flows eastward forming the major drainage of the northern half of Kansas. Typically, the stream meanders through a wide flat sandy river bed; shore vegetation is generally sparse. The river was interrupted by the construction of Cedar Bluff Dam in Trego County in 1951. The reservoir was constructed primarily for irrigation, municipal water, and flood control (11), and at conservation level extends approximately 15 km upstream. During the course of this study, water from the reservoir was used so extensively for irrigation that none was

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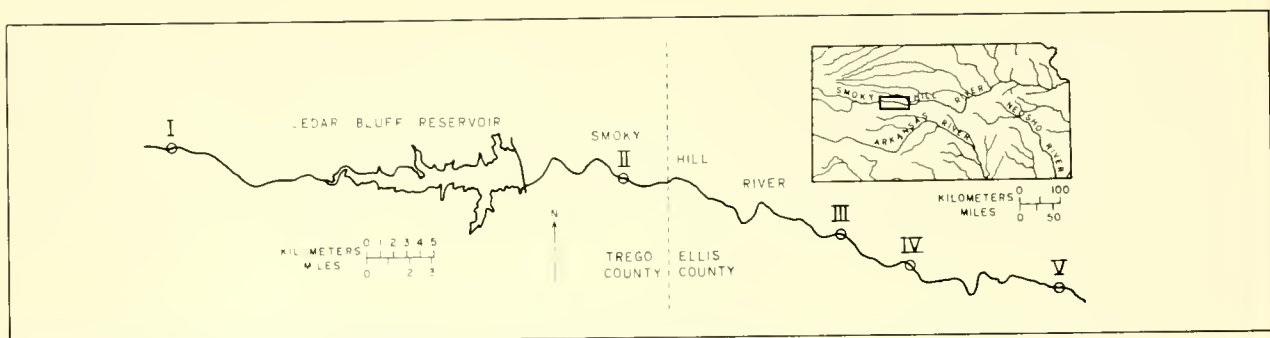


FIGURE 1.—Map of the Smoky Hill River showing the fish sampling sites, Trego and Ellis Counties, west-central Kansas

released into the river below the dam, and conditions were relatively stable for some distance below the dam. The entire volume of the river was the result of springs, seepage, local precipitation, and irrigation runoff below the dam. Stream volume was quite low near the dam, but increased progressively with distance downstream.

SAMPLING STATIONS

Samples were collected at five locations on the river spanning about 66 km in a straight line or about 80 river km. One station was upstream from Cedar Bluff Reservoir, and one was just below the reservoir, upstream from the irrigation region. The other three stations were on the river along the irrigation district.

Fig. 1 shows the general location of the study area; Table 1 gives locations, the size of the stream at each station, and brief descriptions of the sample stations. Each station was located close to a bridge to facilitate sampling; stations were numbered consecutively downstream. The stream water was fairly clear except for a few days following rains. Dissolved oxygen usually was near saturation; water temperature typically varied greatly throughout the day; the pH ranged from 7.6 to 8.3, total alkalinity, from 140 ppm to 220 ppm.

COLLECTION OF SAMPLES

Fish populations were sampled during 1967, 1968, and 1969, using a 230-volt AC, 3,000-watt electro-shocker with two hand-held electrodes. The generator was placed on bridges; a power reel with 110 meters of cord permitted sampling that distance from either side of the bridge.

To compare fish populations, fish from 91 m (100 yd) of stream were collected by two persons using the hand-held electrodes and dip nets and wading upstream in a zig-zag pattern until fish in the entire 91-m section were collected. The electrodes were held in front of the collectors, and stunned fish were taken in dip nets; care was taken to avoid muddying the water. The same 91-m section was sampled each time at each station.

SAMPLES FOR PESTICIDE ANALYSIS

Several species of fishes and some specific fish tissues were analyzed for pesticide residues. Whole bodies of four species (stoneroller, red shiner, plains killifish, and green sunfish) were analyzed. These four species represented different trophic levels and were among the most abundant small species in the area, thus most likely subject to predation by larger animals. Knowledge of residue levels in these species would be useful in detecting problems of food chain concentration. Attempts were made to collect about 25 fish of each species at each station, but often it was not always possible to collect that many. All species were not collected at all stations.

Samples of fish flesh were collected from the most abundant game species, channel catfish, and the two largest species, carp and river carpsucker. These were the ones most likely to be eaten by man; therefore, this tissue was analyzed to detect possible hazards. Attempts were made to obtain flesh from up to 10 individuals per species per station, but frequently this amount could not be collected. Flesh was collected by filleting the fishes and removing the skin from the fillets.

Pesticide residues were checked in gonads to detect possible effects on reproduction. To obtain quantities sufficient for analysis, gonads were collected from carp and river carpsucker, the largest species present. Attempts were made to get gonads from five females and five males from both species at each station during each sampling; it was not possible to collect that quota. Carp usually had sizable gonads most of the year; carpsucker gonads were greatly reduced after mid-summer often making it impossible to get large enough volume for analysis.

The samples were partially processed immediately after being collected. The fishes for whole body analysis were individually measured and weighed together and then frozen on dry ice. The fish flesh and gonad samples were taken from fishes that had been weighed and measured. Flesh and gonads of individual fishes were bagged separately in plastic bags and frozen on dry ice.

TABLE 1.—Description of sampling stations on Smoky Hill River, distance from Cedar Bluff Dam, and the approximate average size of stream

STATION	DESCRIPTION OF SAMPLING STATION ¹	DISTANCE FROM CEDAR BLUFF DAM	APPROX. AVERAGE SIZE OF STREAM AT NORMAL LOW FLOW		STREAM FLOW	
			WIDTH (M)	DEPTH (M)	(CMS)	(CFS)
1	In Trego County, stream very shallow and open with a sand bottom; no vegetation on stream's edges because it meandered over a wide river bed of sand through a short grass prairie.	30.1 river km upstream (26.1 km straight line) and about 13.5 km from end of reservoir	5.5	0.06	0.08	3
2	In Trego County, stream shallow with sand bottom; small overhanging trees and bushes at edges; some of the bank undercut beneath root masses.	10.6 river km downstream (7.6 km straight line)	6.2	0.16	0.31	11
3	In Ellis County, north edge of the stream bordered part way by a bluff and the rest of the edge by sand with a few scattered trees; bottom part sand and part smooth bedrock; some washed out deep spots (about 0.6 m) with silt bottom.	31.0 river km downstream (24.1 km straight line)	10.0	0.19	0.59	21
4	In Ellis County, part of stream bordered by a bluff; the rest of the edge bordered by gravel or sand with a few small trees; the bottom, sand or gravel with silt in the few deeper holes (1 m) under brush piles.	36.8 river km downstream (29.0 km straight line)	9.7	0.17	0.65	23
5	In Ellis County, edges bordered by a few trees with open sand or gravel; bottom with areas of gravel and of sand with a few holes (1 m) under root masses and brush piles.	50.2 river km downstream (40.1 km straight line)	10.0	0.17	0.65	23

¹ Summerfelt (14) provides more details on the sampling areas.

In the laboratory each sample was thawed and homogenized in a blender. For whole-body analysis individuals of one species from one station were pooled to make a sample. The sample of flesh consisted of a pooling of equal weights of flesh taken from each individual of one species to prevent larger fish from biasing the sample. Gonads were treated similarly, keeping sexes separate. After each pooled sample was homogenized, a subsample was taken for residue extraction.

RESIDUE EXTRACTION AND ANALYSIS

A 10-g subsample was placed in an omni-mixer with 50 ml of redistilled hexane and sufficient anhydrous sodium sulfate to take up the water. The mixture was blended at high speed 1 to 2 minutes. The solution was then decanted through #43 Whatman filter paper into a 100-ml suction flask. The residue was extracted with two additional portions of hexane as described above; extracts were filtered and combined in a suction flask. The container, filter paper, and contents were washed with a final portion (10 ml) of hexane. The total hexane extract was transferred to a round-bottom flask for concentration under vacuum at 35° - 40° C to 2 - 3 ml of hexane. The concentration was transferred quantitatively to a 15-ml centrifuge tube using small portions of hexane totaling 5 ml. An aliquot was used for cleanup and gas chromatographic analysis.

For the cleanup procedure a silica-gel chromatography microcolumn was prepared by packing a plug of glass wool loosely into a disposable pipet, about 4 cm from the tip, and then adding 1 g of high purity Silica Gel 950 (60-200 mesh). Prior to column chromatography, solvent extract was evaporated to 1 ml. For partial deactivation of silica gel, the 1 ml of concentrated extract used for charging the column was saturated with 5 μ l of distilled water and transferred quantitatively to the column. It was allowed to percolate through the column at 1-2 ml per minute. The walls of the column were rinsed with small portions of hexane. When the solvent reached the top of the silica gel, elution with the desired eluting solvent was begun.

The eluting solvents were 2%, 7%, and 70% benzene in hexane, 100% benzene and 8% ethylacetate in benzene. The eluate was collected in a 15-ml graduated centrifuge tube. The eluates were concentrated separately to 1 ml, by a stream of nitrogen just before gas chromatography (8,9).

The analyses were performed with RSCO Model #600 series and Barber-Coleman gas chromatographs, both equipped with electron capture detector. Operating conditions were as follows:

Column: 6' glass, packed with 3% DC-11 on 60 to 80 mesh
silinized Gas Chrom P
Temperatures: Column 200° C
Detector 220° C
Injector 240° C
Carrier gas: Nitrogen at a flow rate of 36 ml/min
Volume injected: 4 µl of the extract in hexane

Each sample was analyzed for these organochlorine pesticides: endrin, aldrin, dieldrin, heptachlor, heptachlor epoxide, *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD. The analytical procedure was capable of detecting residues as low as 0.01 ppm of the above pesticides. Insecticide residues were not corrected for recovery since recovery from fortified samples was essentially 100%.

Results and Discussion

FISH POPULATION

During the 3 years, 24 species of fishes were collected from the 5 stations. Table 2 lists these species along with the percent of times they were taken at each station, based on the total number of sample collections at each station. The mean and range in number of individuals collected are also presented. The means are expressed as logarithmic means because most samples contained few individuals of a species with an occasional great number. The species names used are accepted common names established by the American Fisheries Society (1). Fifteen species are considered common in the study area: stoneroller, carp, red shiner, sand shiner, suckermouth minnow, bluntnose minnow, fathead minnow, creek chub, river carpsucker, black bullhead, channel catfish, plains killifish, green sunfish, orangespotted sunfish, and orangethroat darter. All 15 species are rather widely distributed in Kansas (2).

The means and ranges of the number of species and species diversity for each station during the 3 years of this study are presented in Table 3. Species diversity was calculated with the following formula which MacArthur and MacArthur (12) applied to diversity in animal populations:

$$\text{Species Diversity} = - \sum P_i \ln P_i$$

where P_i is the portion or fraction of the number of individuals of species i to the total number of individuals of all species collected and $\ln P_i$ is the natural logarithm of that portion. Then the antilog was taken to express the value in a species equivalent. The species diversity index shows the degree of equality distribution of individuals among the different species collected. If exactly the same number of each species was collected, species diversity would equal the number of species. In general, stable communities have high species diversities, and unstable communities or polluted environments have low species diversities.

Fig 2 shows the trends of numbers of fish species and species diversities over the 3 years at all stations. The stream conditions at Station 1 varied widely, as reflected by the erratic pattern of number of species and species diversities. Stations 2 through 5 showed fairly consistent and similar numbers of species and species diversities, indicating a relatively stable situation.

PESTICIDE RESIDUES

The results of 393 pesticide analyses are given in Table 4 for Stations 1 through 5, respectively. The data for 1967, 1968, and 1969, were summarized together since residue patterns were generally similar for each of the 3 years.

Residues of endrin, aldrin, and heptachlor were not detected in any sample. Dieldrin was detected in a small percentage (15%) of the samples, most of these had only trace (<0.001 ppm) amounts. Heptachlor epoxide was detected in only three samples in trace amounts. Residues of DDT and its metabolites were most frequently detected, with DDE being found in most samples and the other compounds found in only a few samples. In most cases, residue amounts were very low, several hundredths of a part per million.

Biological magnification was not shown in the whole body analysis of small species, since no trend with respect to trophic level of fish species was evident. Residues were low, and these species did not appear to pose a threat to larger predators.

Residues in edible flesh samples were at very low levels, the highest being 1.66 ppm DDE in one sample of channel catfish meat. All samples were considered safe for human consumption, being well below the FDA tolerance levels.

The gonads tended to have the most residues, especially carp testes. Whether or not residues in gonads affected the reproductive success is unknown. Fish populations appeared to be consistent throughout the study with young of all the common species consistently appearing in collections.

Our results compared with those of the National Pesticides Monitoring Program indicate that pesticide residues in fishes of the Smoky Hill River region are among the lowest in the Nation. Henderson *et al.* (4,5) showed DDT and its metabolites in 99% of the samples at levels up to 45 ppm for 1967 and 100% for 1969 with levels up to 57.8 ppm. Seventy-five percent of all our samples had DDT and its metabolites with 0.10 ppm the highest for whole body, 1.66 ppm the highest (second highest was 0.11 ppm) for flesh, and 1.96 ppm the highest (second highest was 0.61 ppm) for gonads. The whole

TABLE 2.—*The logarithmic mean and range (in parenthesis) of numbers of fishes collected per sampling and the percent of times that each species was collected during 1967, 1968, and 1969*

SPECIES	STATION 1		STATION 2		STATION 3		STATION 4		STATION 5	
	MEAN AND RANGE ()	PERCENT TIMES COLLECTED	MEAN AND RANGE ()	PERCENT TIMES COLLECTED	MEAN AND RANGE ()	PERCENT TIMES COLLECTED	MEAN AND RANGE ()	PERCENT TIMES COLLECTED	MEAN AND RANGE ()	PERCENT TIMES COLLECTED
Gizzard shad	0.1 (0-2)	9	0.03 (0-1)	5	0.1 (0-1)	15	0.1 (0-7)	5	0	0
Stoneroller	4.0 (0-38)	82	51.4 (1-354)	100	43.1 (3-182)	100	179.7 (33-1678)	100	86.5 (9-341)	100
Carp	2.9 (0-96)	45	1.7 (0-10)	71	2.3 (0-21)	90	2.2 (0-24)	65	0.3 (0-7)	17
Plains minnow	0.9 (0-43)	27	0.2 (0-2)	19	0	0	0.04 (0-1)	5	0.1 (0-1)	8
Red shiner	3.2 (0-250)	63	142.5 (25-498)	100	93.7 (16-528)	100	292.4 (76-1461)	100	159.0 (47-290)	100
Sand shiner	14.9 (0-662)	82	133.6 (25-460)	100	125.1 (5-634)	100	267.5 (60-1617)	100	138.5 (23-586)	100
Suckermouth minnow	0.4 (0-12)	27	1.6 (0-54)	52	0.4 (0-4)	25	17.9 (0-139)	90	34.0 (7-137)	100
Bluntnose minnow	0	0	46.1 (7-337)	100	98.6 (14-363)	100	163.1 (18-1129)	100	15.8 (3-63)	100
Flathead minnow	2.8 (0-32)	72	17.6 (1-70)	100	34.0 (3-314)	100	41.5 (6-421)	100	3.8 (0-57)	83
Creek chub	0.3 (0-3)	18	13.4 (3-65)	100	8.4 (2-89)	100	8.0 (0-30)	95	4.4 (0-31)	8
River carpsucker	6.1 (0-323)	63	0.8 (0-9)	33	2.0 (0-61)	45	1.5 (0-27)	45	1.0 (0-13)	58
Black bullhead	0	0	2.2 (0-9)	81	1.4 (0-18)	65	0.5 (0-2)	50	0.3 (0-5)	17
Yellow bullhead	0	0	0.1 (0-2)	14	0.04 (0-1)	5	0.04 (0-1)	5	0	0
Channel catfish	0.4 (0-10)	18	1.4 (0-8)	57	2.5 (0-26)	65	1.5 (0-61)	80	2.4 (0-22)	25
Stonecat	0	0	0.1 (0-1)	10	0.04 (0-1)	5	1.4 (0-7)	65	0.9 (0-11)	50
Flathead catfish	0	0	0	0	0.1 (0-1)	10	0.4 (0-4)	30	0.2 (0-1)	25
Plains killifish	171.1 (22-2493)	100	43.4 (7-204)	100	20.6 (0-226)	95	86.5 (23-452)	100	50.9 (2-453)	100
White bass	0.4 (0-7)	18	0	0	0	0	0	0	0	0
Green sunfish	0.3 (0-2)	27	42.8 (10-89)	100	15.0 (0-75)	95	18.9 (3-100)	100	5.4 (0-26)	8
Orangespotted sunfish	0.1 (0-1)	9	0.9 (0-7)	52	3.3 (0-24)	90	3.4 (0-29)	80	0.7 (0-6)	50
Bluegill	0	0	0.1 (0-1)	19	0	0	0	0	0	0
Largemouth bass	0	0	0.4 (0-5)	33	0.1 (0-1)	10	0.2 (0-3)	30	0.3 (0-1)	33
Orangethroat darter	0.4 (0-5)	27	1.3 (0-17)	57	0.5 (0-10)	35	7.1 (0-251)	80	2.1 (0-9)	83
Freshwater drum	0	0	0.03 (0-1)	5	0.3 (0-3)	25	0.1 (0-2)	10	0.2 (0-2)	17
Number of sampling periods	11		21		20		26		12	

TABLE 3.—Mean number of species and species diversity of fishes collected per sampling station in the Smoky Hill River during 1967, 1968, and 1969

STATION	1	2	3	4	5
Mean number of species collected	7.0	13.1	12.8	14.4	12.8
Range in number of species collected	1-12	10-17	9-17	9-18	8-16
Mean species diversity	2.55	6.56	6.55	6.33	5.40
Range in species diversity	1.00-4.44	5.18-7.92	3.23-8.56	4.01-8.46	3.44-6.74
Number of sampling periods	11	21	20	20	12

body results for dieldrin residues by Henderson *et al.* (4,5) for 1967-68 showed residues in 75% of the samples with values up to nearly 2 ppm. During 1969 they found

dieldrin in 93% of the samples with levels up to 1.59 ppm. We detected dieldrin in only 15% of all our samples with highest values in the whole bodies 0.04 ppm, in the flesh 0.01 ppm, and in the gonads 0.08 ppm.

Results of this study indicate that fish populations in the Smoky Hill River of western Kansas are relatively "clean" of pesticide residues. This is undoubtedly because of the low pesticide use in this area of traditional dry-land farming. These data form a baseline with which future studies of the fish populations and residue levels can be compared.

See Appendix for chemical names of compounds discussed in this paper.

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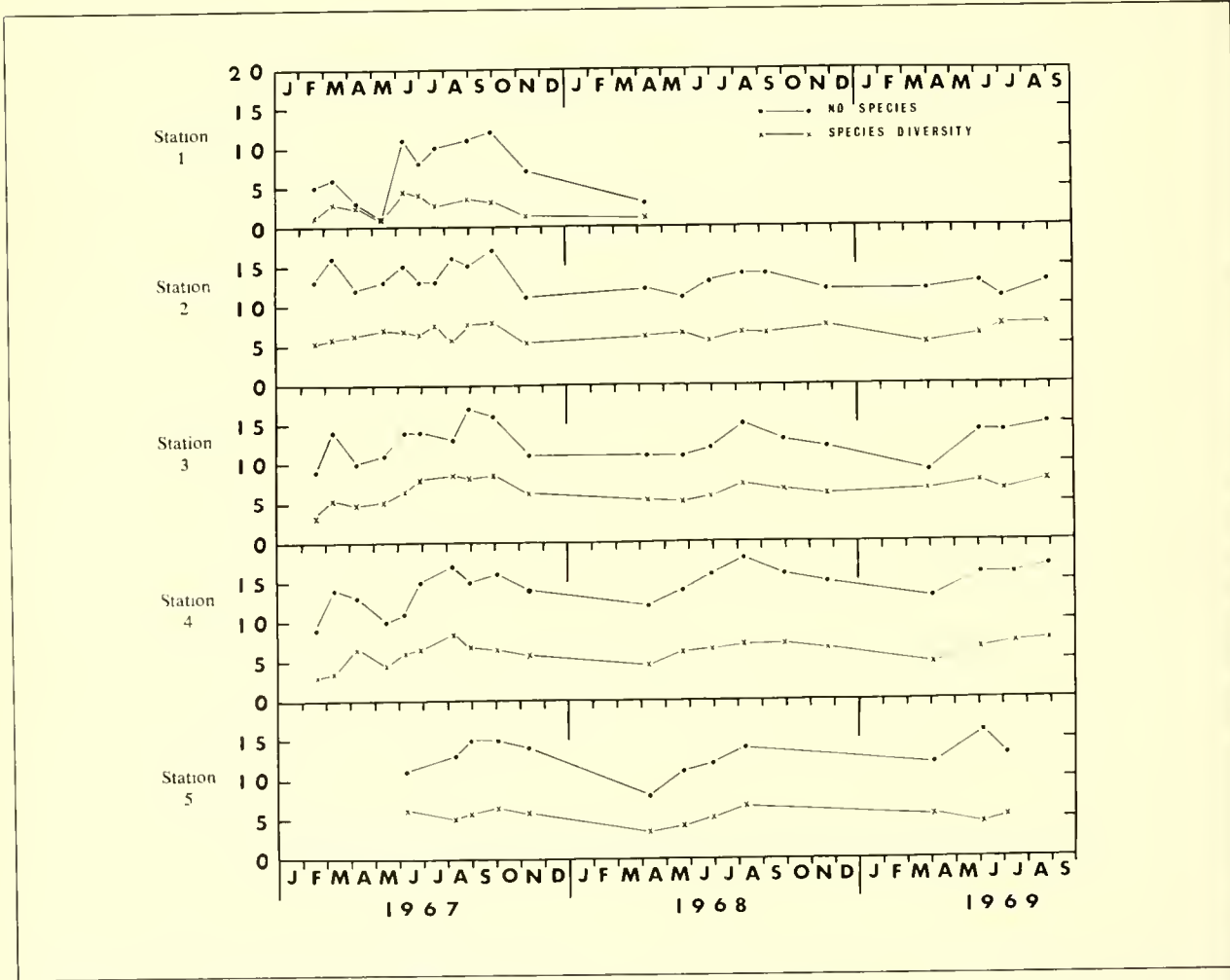


FIGURE 2.—The number of species of fishes collected and species diversity for each sampling period at the five stations during the study, 1967-69

TABLE 4.—Pesticide residues in fish and fish tissues from the Smoky Hill River, Kansas—1967-69
[T = <0.01 ppm]

SPECIES	TISSUES	NUMBER OF SAMPLES ¹	NUMBER OF TIMES DETECTED AND RANGE () IN PPM OF DETECTED RESIDUES ^{2,3}					
			DIELDRIN	HEPTACHLOR EPOXIDE	<i>o,p'</i> - DDT	<i>p,p'</i> - DDT	<i>p,p'</i> - DDE	<i>p,p'</i> - DDD
STATION 1								
Stoneroller	Whole body	1	0	0	0	0	1 (.02)	0
Plains killifish	Whole body	2	0	0	1 (.01)	1 (.02)	2 (.01-.02)	0
Carp	Flesh	2	0	0	0	0	1 (.01)	0
River carpsucker	Flesh	1	0	0	0	0	0	0
Channel catfish	Flesh	1	0	0	0	0	1 (1.66)	0
Carp	Testes	2	0	0	0	0	2 (.02-1.96)	0
	Ovaries	2	0	0	0	0	2 (.04-.06)	0
STATION 2								
Stoneroller	Whole body	14	1 (T)	0	0	0	11 (T-.03)	0
Red shiner	Whole body	15	1 (T)	1 (T)	0	1 (.01)	15 (T-.05)	0
Plains killifish	Whole body	9	0	0	0	0	4 (.01-.02)	0
Green sunfish	Whole body	18	3 (T)	0	0	0	15 (.01-.04)	0
Carp	Flesh	10	1 (T)	0	0	0	8 (T-.04)	0
River carpsucker	Flesh	4	0	0	1 (.01)	0	2 (T-.04)	0
Channel catfish	Flesh	6	0	0	1 (.01)	0	5 (.01-.04)	1 (.03)
Carp	Testes	12	1 (.08)	0	0	0	10 (.01-.58)	1 (.03)
	Ovaries	9	0	0	0	1 (.01)	5 (.01-.03)	0
River carpsucker	Ovaries	1	0	0	0	0	1 (.03)	0
STATION 3								
Stoneroller	Whole body	14	2 (.01)	0	0	0	11 (T-.04)	0
Red shiner	Whole body	14	2 (T)	0	0	1 (.01)	11 (T-.07)	1 (.02)
Plains killifish	Whole body	8	0	0	0	0	4 (T-.01)	0

TABLE 4.—Pesticide residues in fish and fish tissues from the Smoky Hill River, Kansas—1967-69—Continued
[T = <0.01 ppm]

SPECIES	TISSUE	NUMBER OF SAMPLES ¹	NUMBER OF TIMES DETECTED AND RANGE () IN PPM OF DETECTED RESIDUES ^{2,3}					
			DIELDRIN	HEPTACHLOR EPOXIDE	<i>o,p'</i> -DDT	<i>p,p'</i> -DDT	<i>p,p'</i> -DDE	<i>p,p'</i> -DDD

STATION 3—Continued

Green sunfish	Whole body	14	2 (T)	1 (T)	0	0	13 (T-.04)	0
Carp	Flesh	12	2 (T-.01)	0	0	0	8 (T-.03)	0
River carpsucker	Flesh	6	1 (T)	0	1 (.04)	0	2 (T-.01)	0
Channel catfish	Flesh	9	1 (T)	0	0	1 (.01)	6 (.01-.02)	0
Carp	Testes	14	5 (T-.01)	0	0	1 (.01)	14 (T-.14)	0
	Ovaries	13	2 (T)	0	0	0	8 (T-.01)	1 (.01)
River carpsucker	Testes	3	2 (T-.01)	0	0	0	2 (.01-.02)	0
	Ovaries	5	0	0	0	0	1 (.01)	0

STATION 4

Stoneroller	Whole body	14	2 (T)	0	0	0	8 (T-.04)	1 (.01)
Red shiner	Whole body	14	6 (T-.04)	0	0	0	12 (T-.08)	0
Plains killifish	Whole body	10	1 (T)	0	0	0	7 (T-.02)	0
Green sunfish	Whole body	15	2 (T)	0	0	0	11 (.01-.04)	1 (.01)
Carp	Flesh	13	1 (T)	0	0	0	9 (.01-.03)	0
River carpsucker	Flesh	9	2 (T)	1 (T)	0	0	7 (T-.01)	0
Channel catfish	Flesh	10	1 (T)	0	0	0	6 (T-.01)	0
Carp	Testes	14	1 (.01)	0	0	0	14 (T-.08)	1 (.01)
	Ovaries	12	1 (T)	0	0	0	10 (T-.03)	1 (.01)
River carpsucker	Testes	2	2 (.01-.02)	0	0	0	2 (.01-.03)	0
	Ovaries	7	1 (T)	0	0	0	3 (T-.01)	0

STATION 5

Stoneroller	Whole body	4	(T-.03) 0	0	0	0	8 (T-.09)	0
Red shiner	Whole body	2 6	1 1	0	0	0	8 (.01-.04)	0
Plains killifish	Whole body	4 4	1 0	0	0	0	4 (T-.01)	0
Green sunfish	Whole body	1 1	1 1	0	0	1 (.02)	5 (T-.03)	0
Carp	Flesh	8	3	0	1 (.07)	0	2 (.01)	0

TABLE 4.—Pesticide residues in fish and fish tissues from the Smoky Hill River, Kansas—1967-69—Continued
[T = <0.01 ppm]

SPECIES	TISSUE	NUMBER OF SAMPLES ¹	NUMBER OF TIMES DETECTED AND RANGE () IN PPM OF DETECTED RESIDUES ^{2,3}					
			DIELDRIN	HEPTACHLOR EPOXIDE	<i>o,p'</i> - DDT	<i>p,p'</i> - DDT	<i>p,p'</i> - DDE	<i>p,p'</i> - DDD
STATION 5—Continued								
River carpsucker	Flesh		(T-.01) (T)	0	0	0	1 (.01)	0
Channel catfish	Flesh	9	3 (T)	0	1 (.07)	0	5 (T-.02)	1 (.02)
Carp	Testes		(T-.01) (T)	0	0	0	4 (.01-.07)	0
	Ovaries	7	1	0	0	0	3 (.01-.02)	0
River carpsucker	Testes		(T) (T)	0	0	0	1 (.01)	0
	Ovaries	6	2 (T)	0	0	0	1 (.01)	0

¹ For whole body analysis, individuals of one species from one station were pooled to make one sample. The sample of flesh consisted of a pooling of equal weights of flesh taken from each individual of one species; gonads were treated similarly, keeping sexes separate.

² Wet-weight basis.

³ Endrin, aldrin, and heptachlor residues were all zero values.

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Organochlorine Pesticides and Polychlorinated Biphenyls in Black Duck Eggs From the United States and Canada—1971¹

Jerry R. Longcore and Bernard M. Mulhern

ABSTRACT

Black duck (*Anas rubripes*) eggs were collected in 1971 from the Northeastern United States and Canada. All 61 eggs analyzed contained DDE residues; the mean DDE residues for States and Provinces ranged from 0.09 to 5.94 ppm on a wet-weight basis, with mean concentrations exceeding 1.0 ppm in eggs from Maine, New York, New Jersey, and Delaware. The highest DDE concentration, 14.0 ppm, was in an egg from Delaware. DDD and DDT residues averaged <0.5 ppm for each collection area. No mirex residues and only trace amounts of dieldrin and heptachlor epoxide were detected. Of the 61 eggs, 57 contained PCB's; mean PCB residues ranged from <0.05 ppm in samples from Nova Scotia to 3.30 ppm in those from Massachusetts, with trace amounts occurring in nearly half the samples. Mean organochlorine pesticide residues appeared to be lower in the 1971 samples than in those analyzed in an earlier study in 1964.

The shells from the 61 eggs were measured for thickness. Comparisons of the shell thickness of eggs collected in 1971, 1964, and prior to 1940 showed that the average thickness of shells from eggs collected in 1964 (0.321 mm) was significantly less ($P < 0.01$) than that of shells of eggs collected prior to 1940 (0.348 mm) or in 1971 (0.343 mm).

Introduction

A survey of organochlorine pesticides in black duck eggs, completed in 1964, revealed that DDE residues averaged over 4 ppm (wet-weight basis) in eggs from three different States and ranged as high as 10-12 ppm in some eggs (1). With decreasing use and increasing restrictions on DDT in the United States the present survey was made in 1971 to determine whether a downward trend was apparent in residue levels.

Sampling Procedures

In the spring of 1971, two eggs were collected from each of 61 nests along the Atlantic seaboard from Maryland to Nova Scotia by U.S. and Canadian biologists. After collection, eggs were individually wrapped in aluminum foil, packed in a shipping container, and sent to the Patuxent Wildlife Research Center, Laurel, Md. One egg from each clutch was randomly chosen for chemical analysis, and two eggs from each of four clutches (one in Maine, Vermont, Massachusetts, and New Jersey) were analyzed.

Weight, length, and breadth were measured for all eggs. Volume was measured by water displacement, except for cracked eggs. Volumes of cracked eggs were estimated by calculating a regression equation of measured volume (ml) on length (L in cm) x breadth (B in cm) squared (2). The equation for these estimates was $y = 0.506315 LB^2 + 0.924992442$, an equation derived from measurements and volumes of a series of 59 black duck eggs. The difference between measured volume and estimated volume averaged 2.6%. Residue levels that were probably biased upwards from a reduction of moisture or lipids in incubated eggs or cracked eggs were corrected by assuming specific gravity as 1.0 and basing concentration (ppm) on egg volumes. For the cracked eggs ($N=3$), residue values reported herein are conservative assuming chemicals were lost with lipids.

Eggs were cleaned of debris before opening at the shell equator. The stage of embryonic development was noted, and egg contents were then frozen until chemical analysis. Shells were washed and air-dried to constant weight, then measured at the shell equators with a Starrett 1010M micrometer graduated in units of 0.01 mm.

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A statistical comparison of the 1964 and 1971 eggshell thickness was made by analysis of variance with weighting of disproportionate subclasses (3). Student's t-test was used to compare means of shell thickness of eggs collected prior to 1940 and eggs collected in 1964.

Analytical Procedures

Eggs were analyzed individually for residues of organochlorine pesticides and polychlorinated biphenyls (PCB's). Each egg was mixed in an Omni-mixer and a 20-g aliquot was removed for analysis. The 20-g aliquot was ground with anhydrous sodium sulfate and extracted for 7 hours with hexane in a Soxhlet apparatus. The extract was cleaned up, and the pesticides in half the clean extract were separated and removed in four fractions from a thin layer (TL) plate according to the procedure of Mulhern *et al.* (4). The other half of the clean extract was used for PCB analysis.

All four TL fractions were analyzed separately by electron capture gas chromatography on a 4% SE-30/6% QF-1 on 100/120 Gas Chrom Q column; DDT residues in fraction III (and occasionally fraction IV) were confirmed on a 3% QF-1 column. The operating parameters for the SE-30/QF-1 and QF-1 columns are given in Table 1. Average recovery by this method is 85-96%, and the limit of detection is approximately 0.05 ppm for DDE, DDD, DDT, and dieldrin, and 0.01 ppm for heptachlor epoxide. Residues were not corrected for percent recovery.

TABLE 1.—Chromatographic operating conditions (using electron capture detection)

	COLUMNS, GLASS 1/4" O.D.	
	A	B
Column length	4 ft	6 ft
Liquid phase	4% SE-30/6% QF-1	3% QF-1
Support	Gas Chrom Q	Gas Chrom Q
Mesh size	100/120	100/120
Column flow rate	60 ml/min	100 ml/min
Purge	40 ml/min	—
Gas	5% methane/argon	nitrogen
Temperature	200° C	170° C
Retention time of dieldrin	13.5 min	17.5 min

PCB's were determined by the method of Mulhern *et al.* (5). In this method, the clean extract is evaporated to dryness, and the residue is reacted with chromic acid-acetic acid oxidative reagent in a water bath for 1/2 hour. The reacted mixture is then extracted with two successive portions of 10 ml of hexane, which are pooled, washed with water until neutral to hydriion paper, filtered through drierite, and concentrated to 100 μ l for TLC.

Analysis for PCB's by TLC was accomplished by spotting 1, 5, and 10 μ l dilutions of oxidized extract and 1, 3, and 5 μ g quantities of Aroclor 1254® on AgNO₃ incorporated Al₂O₃ plates. Plates were developed in a solvent mixture of 5% benzene in hexane, removed, dried, and then exposed to germicidal UV lights until completely color developed. Semiquantitation was accomplished by comparing a sample spot to Aroclor 1254. The limit of sensitivity was 1.00 ppm.

Results and Discussion

The results of analyses are shown in Table 2. DDE was detected in all eggs; residues in individual eggs ranged from a trace (<0.05 ppm) to 14.0 ppm on a wet-weight basis. The mean DDE residues from States or Provinces ranged from 0.09 in Nova Scotia eggs (N=2) to 5.94 ppm in Delaware eggs (N=5). DDE in eggs from Maine, New York, New Jersey, and Delaware averaged greater than 1.0 ppm. DDD occurred in 31 of 61 eggs, in 16 as trace amounts but as high as 0.32 ppm in one. DDT was present in 55 of 61 eggs, in 7 as trace amounts and with a high value of 1.19 ppm. Average DDT residues did not exceed 0.42 ppm for any State or Province. Dieldrin was present in 59 of 61 eggs, in 35 as trace amounts and with a high value of 0.81 ppm. State or Province dieldrin means ranged from 0.022 to 0.208 ppm. Heptachlor epoxide occurred in 46 eggs, in 10 as trace amounts; the high value was 0.16 ppm. The highest mean residue for any State or province was 0.14 ppm in the New York sample. No mirex was detected.

PCB's were detected in 57 of the 61 eggs; 28 eggs contained trace amounts, <1.0 ppm. PCB means ranged from <0.05 ppm in Nova Scotia to 3.30 ppm in Massachusetts. The highest PCB residue was 6.9 ppm. Lipid weight averaged 14.1% of the fresh weight of the egg contents. Residues in the two eggs from each of four different clutches were similar except for the pair analyzed from New Jersey (Table 3).

Average shell thickness of fresh eggs plus eggs incubated less than 1 week was 0.343 mm (N=52). Shells of eggs incubated for longer periods (N=9) averaged 0.349 mm. Thickness at the equator of all 61 shells averaged 0.344 mm, with a range of 0.30-0.40 mm.

Egg residues found in this survey are not strictly comparable to those found in the 1964 survey, because of disproportionate sample sizes from the different States and Provinces. However, DDE residues measured in the present survey were generally lower than those in 1964. One exception to this general pattern was the DDE residues in the five 1971 eggs from Delaware which had a mean residue of 5.9 ppm of DDE and a

range of 2.0-14.0 ppm. In the 1964 survey, the DDE residues in six Delaware eggs averaged 1.7 ppm (range 0.05-2.3 ppm). DDD and DDT residues were also lower in the present survey (Table 2).

In the 1964 survey, DDE averaged over 4.0 ppm in eggs from three different States: New York, 4.4 ppm; New Jersey, 4.6 ppm; and Massachusetts, 5.5 ppm (1), with DDE residues in some eggs from each of these States exceeding 10 ppm.

Dieldrin and heptachlor epoxide occurred more frequently in eggs collected in the present survey than in those of the 1964 survey, but individual eggs contained similarly low concentrations in both years. The increased frequency of occurrence is probably related to the greater sensitivity for quantitation. In the 1964 survey, the sensitivity for heptachlor epoxide and dieldrin was 0.2 ppm; in the present survey, the sensitivity for heptachlor epoxide was 0.01 ppm and for dieldrin, 0.05 ppm.

TABLE 2.—Organochlorine pesticide and PCB residues in black duck eggs from the United States and Canada—1971

COLLECTION AREA	NUMBER OF EGGS		RESIDUES IN PPM, WET WEIGHT BASIS ¹					
			p,p'-DDE	p,p'-DDD	p,p'-DDT	DIELDRIN	HEPTACHLOR EPOXIDE	PCB's
CANADA								
Nova Scotia	2	Mean	0.090	—	0.025	0.025	0.0025	0.05
		Median	0.09	—	0.025	0.025	0.0025	0.025
		Range	0.07-0.11	(2ND)	(2T)	(2T)	(1ND,1T)	(2T)
New Brunswick	3	Mean	0.173	—	0.060	0.025	0.002	0.333
		Median	0.19	—	0.05	0.025	0.00	0.5
		Range	0.09-0.24	(3ND)	0.05-0.08	(3T)	(2ND,1T)	(1ND,2T)
Quebec	5	Mean	0.790	0.010	0.254	0.040	0.006	1.1
		Median	0.77	0.00	0.25	0.025	0.005	1.0
		Range	0.22-1.50	(3ND,2T)	0.06-0.60	0.10(4ND)	0.01-0.02(3ND)	1.0-2.0(1T)
Ontario	5	Mean	0.820	0.010	0.254	0.030	0.011	1.50
		Median	0.70	0.00	0.28	0.025	0.01	0.50
		Range	0.28-1.49	(3ND,2T)	0.07-0.41	0.05(4T)	0.01-0.02(1T)	0.5-3.00(2T)
UNITED STATES								
Maine	3	Mean	1.013	0.016	0.420	0.035	0.003	0.67
		Median	0.51	0.025	0.42	0.025	0.00	0.50
		Range	0.29-2.24	(1ND,2T)	0.07-1.19(1ND)	0.08(1ND,1T)	0.01(2ND)	1.00(2T)
New Hampshire	3	Mean	0.480	—	0.211	0.025	0.010	0.50
		Median	0.53	—	0.025	0.025	0.006	0.50
		Range	0.19-0.72	(3ND)	0.61(1ND,1T)	(3T)	0.006-0.02(1T)	0.50(2T)
Vermont	9	Mean	0.513	0.002	0.096	0.022	0.006	0.611
		Median	0.48	0.00	0.09	0.025	0.005	0.50
		Range	0.15-1.00(1T)	(8ND,1T)	0.07-0.27(2ND)	(1ND,8T)	0.007-0.02(3ND,2T)	1.00-2.00(2ND,5T)
Massachusetts	5	Mean	0.924	0.044	0.222	0.208	0.014	3.30
		Median	0.65	0.025	0.14	0.07	0.010	3.00
		Range	0.28-1.90	0.07-0.10(1ND,2T)	0.09-0.69(1ND)	0.07-0.81(2T)	0.01-0.05(2ND)	1.00-6.90
Connecticut	1	Mean	0.210	0.025	0.08	0.025	0.005	0.50
		Median	—	—	—	—	—	—
		Range	0.21	(1T)	0.08	(1T)	(1T)	(1T)
New York	3	Mean	1.230	0.123	0.236	0.10	0.040	1.50
		Median	0.46	0.05	0.16	0.10	0.03	2.00
		Range	0.46-2.70	0.05-0.32(1ND)	0.05-0.50	0.09-0.11	0.01-0.08	0.50-2.00
New Jersey	8	Mean	1.971	0.084	0.382	0.061	0.018	1.434
		Median	1.75	0.055	0.36	0.07	0.015	1.00
		Range	0.97-3.20	0.06-0.23(1ND,2T)	0.12-0.78	0.06-0.09(2T)	0.01-0.04(1T)	1.00-4.0(1T)
Delaware	5	Mean	5.940	0.120	0.205	0.124	0.012	0.90
		Median	4.30	0.15	0.10	0.05	0.02	0.50
		Range	2.00-14.00	0.15-0.23(2T)	0.10-0.53(1ND,1T)	0.05-0.44(2T)	0.02(2ND)	1.00-2.00(3T)
Maryland	9	Mean	0.298	0.030	0.062	0.128	0.037	0.50
		Median	0.27	0.025	0.07	0.12	0.01	0.50
		Range	0.14-0.49	0.05-0.11(4ND,2T)	0.07-0.12(3T)	0.05-0.39(3T)	0.01-0.16(2T)	1.00(1ND,7T)

NOTE: ND = not detected; T = trace = <0.05 ppm for DDE, DDD, DDT, and dieldrin and <0.01 ppm for heptachlor epoxide and <1.00 ppm for PCB's; ranges exclude ND and T values, which occurred in number of samples shown in parentheses. All values were used in computing means and medians; for means, trace values were used as one-half the stated "less than" value and ND was used as zero.

¹ Mirex was not detected in any egg.

In 1971, mean shell thickness (0.343 mm) of the 52 eggs incubated less than 1 week was 1.4% less than the mean thickness (0.348 mm) of 34 black duck eggshells collected between 1876 and 1939 in the Northeast United States and Canada (E. E. Klaas and H. M. Ohlendorf, Patuxent Wildlife Research Center, *personal communication*). These 34 eggs also were either fresh or incubated less than 1 week. Eggs with developed embryos were excluded from thickness computations because shell thickness may change with embryonic development (6).

In the 1964 survey, two eggs were collected from each clutch. One egg from each of these clutches was analyzed for residues, but shells were not saved. Shells from the remaining eggs were measured later. Average thickness of 37 eggs from Maine, New York, Delaware, Massachusetts, and Vermont was 0.321 mm, which is significantly less ($P < 0.01$, $F_{0.01}$ with 1 & 50 df = 7.17; ANOV $F = 7.16$) than the average thickness of shells of 23 eggs (2 eggs with developed embryos excluded from computations) collected in the same States in 1971 (0.340 mm). In both surveys most eggs were collected in the same general geographic area in each of the five States. The average shell thickness of the 1964 eggs (0.321 mm) was also significantly less ($P < 0.01$) than the mean thickness (0.348 mm) of the black duck eggshells collected between 1876 and 1939. These data suggest that shell thinning may have lowered productivity for individual hens through shell cracking and contributed to the declining ratios of immature to adult birds in the years of the early 1960's (7). Dietary DDE causes shell thinning among captive black ducks (7) and predisposes eggs to a higher incidence of shell cracking (8).

Although the 1964 and 1971 samples were not strictly comparable, it appears that organochlorine residues in black duck eggs are now lower than those reported in 1964. These lower residues may reflect the reduced use

of DDT in urban and agricultural areas in the 1960's and discontinuance of the practice of spraying marshlands with DDT for insect control. In Arizona, reductions in DDT and DDE residues have been noted in beef fat and alfalfa 2 years following a moratorium on DDT use (9). Similarly, dieldrin and DDE levels in shag eggs (*Phalacrocorax aristotelis*) collected between 1964 and 1971 have declined significantly during this period in Eastern Britain following restrictions on pesticide use (10).

Acknowledgment

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See Appendix for chemical names of compounds discussed in this paper.

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TABLE 3.—Residues in pairs of eggs from each of four different clutches

COMPOUND	RESIDUES IN PPM, WET WEIGHT BASIS ¹								
	CLUTCH 1 MAINE		CLUTCH 2 VERMONT		CLUTCH 3 MASSACHUSETTS		CLUTCH 4 NEW JERSEY		
	Egg No.:	I	II	I	II	I	II	I	II
p,p'-DDE		0.29	0.33	0.15	0.22	1.26	1.05	1.70	2.82
p,p'-DDD		T	T	ND	ND	0.07	0.15	T	0.14
p,p'-DDT		ND	T	0.09	0.07	0.69	0.65	0.27	0.61
Dieldrin		0.08	0.08	T	T	T	T	0.09	0.14
Heptachlor epoxide		ND	ND	T	ND	0.05	0.03	0.01	0.06
Polychlorinated biphenyls		T	1.0	T	T	1.0	2.0	1.0	2.0

NOTE: ND = Not detected.

T = trace = <0.05 ppm for DDE, DDD, DDT, and dieldrin; <0.01 for heptachlor epoxide; and <1.00 for PCB's.

¹ Mirex was not detected in any egg.

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Mercury, Lead, Cadmium, and Arsenic Residues in Starlings—1971

William E. Martin¹ and Paul R. Nickerson²

ABSTRACT

Starlings (Sturnus vulgaris) collected in 1971 at 50 sites throughout the contiguous United States were analyzed for mercury, lead, cadmium, and arsenic residues. Except for one sample containing 0.62 ppm, mercury levels were generally well below 0.5 ppm with 76% at 0.05 ppm or less. Lead levels ranged from 0.12 to 6.6 ppm; cadmium residues ranged from "not detected" to 0.24 ppm with 72% at 0.05 ppm or less; and arsenic levels were quite low with only one sample (0.21 ppm) exceeding 0.05 ppm.

Introduction

This report presents the 1971 residue findings for mercury, lead, cadmium, and arsenic in starlings (*Sturnus vulgaris*) collected at 50 of 53 preselected sites throughout the contiguous United States in November and December 1971. A description of this monitoring program and results of the 1970 collections analyzed for mercury and lead were reported by Martin (1); these 1970 results provide general baseline information. In 1971, cadmium and arsenic residue determinations were added and the number of sampling sites was increased from 25 to 53 (Fig. 1).

Sampling Design and Collection Procedures

Sampling site locations are shown in Fig. 1. The 53 sampling sites used in the 1971 collection include 25 sites initially established in 1970 (1) to determine the suitability of starlings as indicators of environmental lead

residue levels, and 28 new sites selected to broaden the monitoring design (Fig. 1 and Table 1). The sites were chosen to reflect varying environmental conditions representing broad geographic areas and including differing degrees of human activity and related pollution sources. In 1970, a larger sample was needed to assess quickly levels of mercury in starlings; therefore, the 125 samples taken for organochlorine insecticide residue determination were also analyzed for mercury, as reported by Martin (1). In 1971, mercury analyses were included with the other heavy metals.

Samples for heavy metal residue determination were not obtained from 3 of the 53 preselected sites (Albany, N.Y.; Worland, Wyo.; and southern Indiana) because of scarcity of starlings during the prescribed collection period.

Each sample consisted of a "pool" of 10 starlings collected by means other than lead shot or poison. The 10-bird pools were coded, wrapped in aluminum foil, placed in polyethylene bags, and frozen immediately for shipment to the laboratory for analysis. Collections were made by field personnel of the Division of Wildlife Services of the Bureau of Sport Fisheries and Wildlife.

Analytical Procedures

All residue determinations were done by the Section of Chemical Research and Analytical Services, Denver Wildlife Research Center, Colo., Bureau of Sport Fisheries and Wildlife. Analytical methods were developed and/or refined by personnel of the Center.

The birds were prepared by skinning and removing beaks, feet, and wings at the first joint out from the

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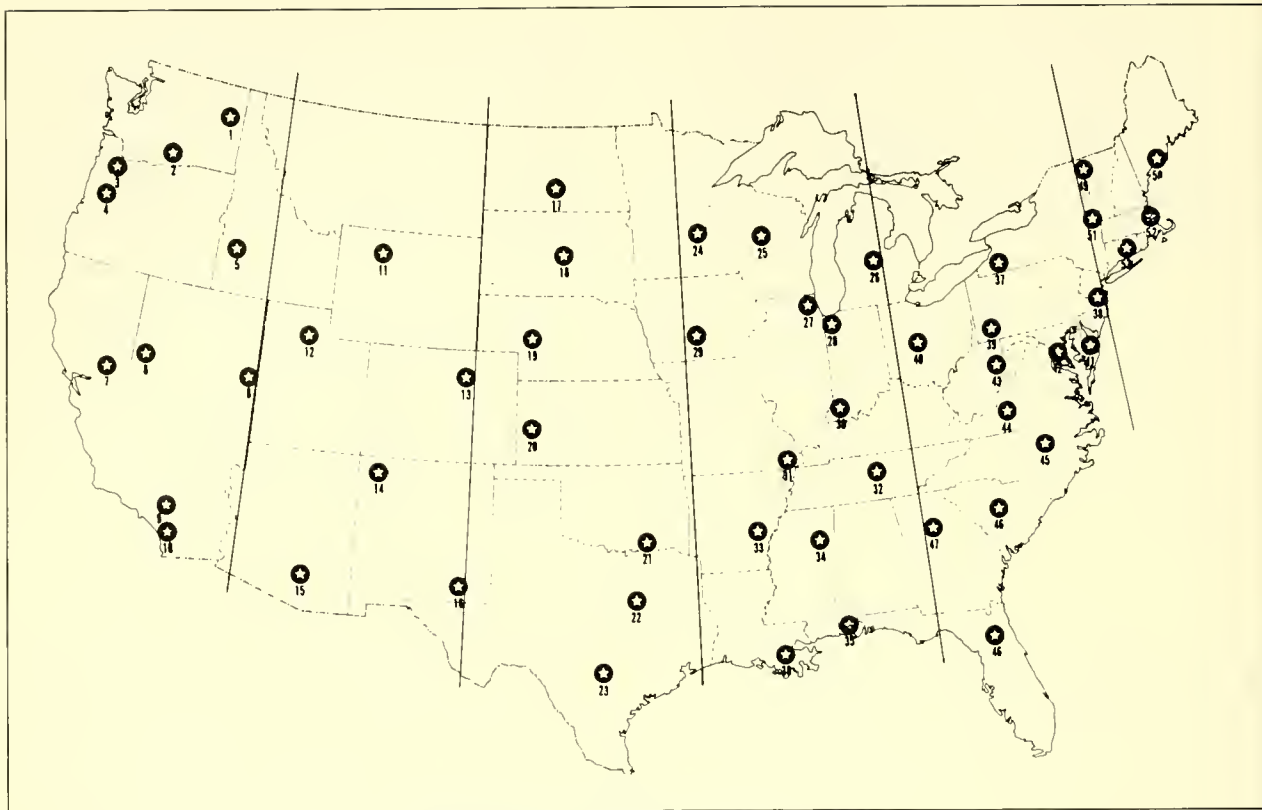


FIGURE 1.—Starling monitoring sites—1971

body. The removed parts were discarded, and each 10-bird sample was ground together in a Hobart food chopper. Atomic absorption spectrophotometric methods yielded the following minimum levels of analytical sensitivity: mercury—0.01 ppm; lead—0.1 ppm; cadmium—0.05 ppm; and arsenic—0.01 ppm.

Two aliquots were taken from each 10-bird sample: A 1-g aliquot was used for mercury determination via flameless atomic absorption (2). A 15-g aliquot was used for determination of arsenic, cadmium, and lead residues via acid digestion and atomic absorption (W. H. Robinson, R. A. Wilson, M. Mohn, G. Ise, M. Robbin, and C. Church. 1972. Method of analyses for arsenic, lead, and cadmium from animal tissue, Denver Wildlife Research Center, *personal communication*).

MERCURY ANALYSIS

Mercury analysis was carried out using the method of Okuno, Wilson, and White (2). The 1-g aliquot of wet homogenized sample was desiccated over P_2O_5 for 14 hours. The dry sample then was burned with oxygen in a combustion flask, and the combustion products were collected in dilute HCl. After the acid solution was reduced with a 20% hydroxylamine hydrochloride solution, the mercury was extracted from the solution by

amalgamating it onto a silver wire. The silver wire then was placed in an absorption cell of the spectrophotometer and heated, by its own electrical resistance, to volatilize the mercury. Spectrophotometric responses were recorded, and micrograms of mercury in the samples were read from a standard curve. A fresh standard curve for 0.001 to 0.2 μg mercury was prepared daily.

Determination was made with a Jarrell-Ash Model 82-360 spectrophotometer fitted with a 10-mv percent absorption recorder. Instrument conditions were:

Wavelength—253.7 nm
Slit—100 μm
Source—Hg hollow-cathode tube (type 22847)
Air—2 liters/min

The average recovery for the method (2) was 91.8% $\pm$ 11%, and the coefficient of variation was 16.9% as determined by tissue samples spiked with organic mercury compounds at levels of 0.01 to 1.0 ppm mercury. A coefficient of variation of 22.0% was found for 68 unspiked tissue samples. A coefficient of variation of 25.9% for the method was determined for 66 duplicates obtained from the 1971 starling and fish monitoring collections made by the Bureau of Sport Fisheries and Wildlife. The results of fish monitoring are reported separately.

TABLE 1.—*Lead, mercury, cadmium, and arsenic residues in starlings, United States—1971*

STATE	CITY OR COUNTY	SAMPLING SITE NUMBER	TYPE OF SITE ¹	RESIDUES IN PPM (WHOLE BODY, WET-WEIGHT)			
				MERCURY	LEAD	CADMIUM	ARSENIC
Alabama	Mobile	35	U	0.07	1.70	<0.05	0.01
Arizona	Phoenix	15	R	0.01	1.80	0.11	0.01
Arkansas	Stuttgart	33	R	0.06	2.00	0.10	0.01
California	Bakersfield	9	U	0.05	0.67	0.24	0.02
	Duplicate Analysis			0.05	0.64	0.11	0.01
	Los Angeles	10	U	0.01	0.70	0.06	0.02
	Sacramento	7	U	0.04	0.30	<0.05	0.01
Colorado	Greeley	13	R	0.02	0.70	0.05	0.01
Connecticut	Conn. River Valley	53	R	0.03	1.00	<0.05	—
Delaware	Dover	41	R	0.03	1.20	0.09	0.01
Florida	Gainesville	48	R	0.21	0.52	<0.05	0.03
Georgia	Atlanta	47	U	0.03	2.80	<0.05	0.01
Idaho	Boise	5	R	0.07	0.50	<0.05	0.01
Illinois	Chicago	27	U	0.01	5.00	0.06	0.03
Indiana	Evansville	30	R				
	Highland	28	U	0.02	3.40	<0.05	0.02
Iowa	Des Moines	29	U	—	0.20	—	0.01
Kansas	Garden City	20	R	<0.01	1.00	0.05	—
Louisiana	Baton Rouge	36	R	0.05	0.21	<0.05	0.01
Maine	Gray	50	R	0.06	0.45	<0.05	0.04
Maryland	Patuxent	42	U	—	1.10	<0.05	0.01
	Duplicate Analysis			0.01	.90	<0.05	0.02
Massachusetts	Quincy	52	U	0.05	6.60	<0.05	—
Minnesota	Twin Cities	24	U	0.01	1.30	0.08	0.01
Michigan	Lansing	26	U	0.03	1.60	0.12	0.21
Mississippi	Starkville	34	R	0.01	0.26	<0.05	0.03
Missouri	Malden	31	R	0.02	1.20	<0.05	0.01
Nebraska	North Platte	19	R	0.01	2.60	<0.05	0.02
Nevada	McGill	8	R	0.05	0.35	<0.05	0.01
	Reno	6	R	0.02	0.13	<0.05	0.04
New Jersey	New Brunswick	38	U	0.16	2.60	<0.05	0.01
	Duplicate Analysis			0.28	2.60	<0.05	0.01
New Mexico	Carlsbad	16	R	0.05	0.22	<0.05	0.01
	Farmington	14	R	0.02	2.3	0.12	0.01
	Duplicate Analysis			0.03	2.2	0.10	0.01
New York	Albany	51	U				
	Jamestown	37	U	0.01	5.10	0.08	0.02
North Carolina	Raleigh	45	U	0.01	1.10	<0.05	—
North Dakota	Mandan	17	R	0.02	0.24	—	0.01
Ohio	Columbus	40	R	0.06	0.46	0.08	0.01
Oklahoma	Tishomingo	21	R	0.01	0.90	<0.05	—
Oregon	Corvallis	4	R	0.32	0.14	<0.05	0.01
	Wilsonville	3	R	0.62	1.50	0.09	0.02
Pennsylvania	Pittsburgh	39	U	0.06	2.40	<0.05	—
South Carolina	Columbia	46	U	<0.01	1.10	<0.05	—
South Dakota	Pierre	18	R	0.05	0.36	<0.05	0.01
	Duplicate Analysis			0.05	0.32	<0.05	0.01

TABLE 1.—Lead, mercury, cadmium, and arsenic residues in starlings, United States—1971—Continued

STATE	CITY OR COUNTY	SAMPLING SITE NUMBER	TYPE OF SITE ¹	RESIDUES IN PPM (WHOLE BODY, WET-WEIGHT)			
				MERCURY	LEAD	CADMIUM	ARSENIC
Tennessee	Nashville	32	U	0.03	1.30	<0.05	0.01
Texas	Hillsboro	22	R	0.04	0.90	<0.05	0.01
	San Antonio	23	U	0.03	0.50	<0.05	0.02
Utah	Salt Lake City	12	R	0.30	0.80	0.06	0.01
Vermont	Champlain Valley	49	R	0.10	1.50	<0.05	—
Virginia	Blacksburg	44	R	0.01	0.70	<0.05	0.01
Washington	Spokane	1	R	<0.01	0.12	<0.05	0.01
	Yakima	2	R	0.02	0.52	<0.05	0.01
West Virginia	Elkins	43	R	0.01	0.90	0.12	0.01
Wisconsin	Horicon	25	U	0.03	0.54	<0.05	0.02
Wyoming	Worland	11	R				

NOTE: Blank = no sample taken; — = not detected.

¹ U = urban, suburban area; R = rural area.

The method is reported to be capable of determining levels as low as 0.01 ppm from a 1.0-g sample. For this study, this was considered adequate; levels below 0.01 ppm are reported only as being identified below that level.

ARSENIC, LEAD, AND CADMIUM ANALYSES

Digestion for Arsenic, Lead, and Cadmium

The 15-g aliquot of the homogenized wet sample was placed in a Kjeldahl flask and digested with 30 ml of a 1:1 mixture of H_2SO_4 and HNO_3 , adding additional HNO_3 as required to maintain an oxidizing condition. The digestion was continued until SO_3 (white) fumes appeared and the sample turned water clear upon cooling. Then, 10 ml of saturated ammonium oxalate solution was added to the cooled sample, and it was reheated until SO_3 fumes were again evident. After the contents of the flask were again cooled, they were transferred to a 100-ml graduated cylinder and made to 60 ml with water rinsings from the flask. Four milliliters of this digest was equal to 1.0 g of the original sample.

The digest was divided into a 10-ml (2.5 of the sample) aliquot for arsenic determination, a 40-ml (10.0 g of the sample) aliquot for lead and cadmium determination, and a 10-ml (2.5 g of the sample) aliquot was held in reserve.

Arsenic Determination

The method used for arsenic determination was a modification of other methods (W. H. Robinson *et al.*, *personal communication*, 3-6). A 10.0-ml aliquot was placed in a reaction flask to which were added 1 ml of 1% KI, 0.5 ml of 20% $SnCl_2$ in concentrated HCl, and 10.0 ml of a 1:1 HCl- H_2O solution. The mixture was allowed to stand for at least 10 minutes. A rubber

balloon containing 0.5 g of a 50:50 mixture of 20- and 40-mesh arsenic-free zinc was attached to a standard taper male joint of the reaction flask. After flushing the system with helium for about 10 seconds, the zinc was added to the reaction flask by raising and shaking the balloon. The reaction was allowed to proceed for 5 minutes. The contents of the reaction flask then were flushed into the burner of the spectrophotometer.

Determination was made with a Jarrell-Ash Model 82-360 spectrophotometer fitted with a 10-mv percent absorption recorder and 10-cm slot burner. Results were quantitated using a standard curve prepared from known reference solutions of arsenic treated in the same manner as the sample digest. Instrument parameters were:

Wavelength—193.6 nm
Slit—4
Lamp current—18-22 ma (normally at 20)
Fuel—Hydrogen
Oxidizer—Ambient air
Carrier gas—Helium

The average recovery for three sets of three samples spiked at 0.10 ppm, 0.25 ppm, and 0.50 ppm was 82% $\pm$ 8%. The average coefficient of variation for 39 fish and starling duplicate analyses was 18.6%.

Lead and Cadmium Determination

The method for determination of lead and cadmium was developed by the Denver Wildlife Research Center (W. H. Robinson *et al.*, *personal communication*). A 40.0-ml aliquot of the digest was placed in a 250-ml beaker and made to 60 ml with distilled water. To this, 15.0 ml of a 50% NaOH solution and 3 drops of a 1% Thymol blue aqueous solution were added. The pH was adjusted to 5-6 with dilute H_2SO_4 and NaOH. The sample was then quantitatively transferred to a 250-ml

separatory funnel to which was added 10 ml of a 1% sodium diethyldithiocarbamate solution. After shaking, the stoppered container was allowed to sit for 10 minutes or more. Then, 10.0 ml of H₂O-saturated methylisobutylketone (MIBK) was added, and the stoppered funnel was shaken for at least 2 minutes to extract the metals into the organic phase. The MIBK was then collected in a 16-by 125-mm culture tube and centrifuged if solids were present, prior to aspiration into the spectrophotometer.

Separate determinations were made for lead and cadmium with a Perkin-Elmer Model 303 atomic absorption spectrophotometer fitted with a 10-mv percent absorption recorder. Results were quantitated using standard curves prepared from known reference solutions (separate solutions for lead and cadmium) treated in a manner similar to the sample.

Instrument parameters were:

Lamp number	45488—Pb 22933—Cd
Lamp current	8 ma—Pb 10 ma—Cd
Wavelength	283.3 nm—Pb 228.7 nm—Cd
Fuel	C ₂ H ₂
Silt	4
Aspiration rate	4 ml/min

The average recovery for three sets of three samples spiked with lead at 0.1 ppm, 1.0 ppm, and 5.0 ppm was 109% ± 33%. Average recovery for samples spiked with cadmium at 0.05 ppm, 0.25 ppm, and 0.50 ppm was 99% ± 19%. The coefficients of variation were 11.5% for 31 fish and starling duplicate lead analyses and 37.1% for 11 fish and starling duplicate cadmium analyses.

Results and Discussion

MERCURY

In general, the 1971 residue levels of mercury in starlings appear to be quite similar to those reported for 1970 even though the 1970 and 1971 sampling designs differed considerably (1). Uniformly low residue levels were found throughout the Nation except for a few sites (Table 1). Of the 50 samples analyzed, 38 had residue levels of 0.05 ppm or less; of the remaining 12, five exceeded 0.10 ppm: Florida—0.21 ppm; New Jersey—0.22 ppm; Utah—0.30 ppm; and 2 sites in Oregon—0.32 ppm and 0.62 ppm. As in 1970, the highest residue levels were found in birds from Oregon.

Other studies of mercury residues in various fish and wildlife species have revealed levels somewhat higher than those reported here which probably can be attributed to several factors including diet, food chain relationships, sampling methods, and sampling period.

Wishart (7) reports considerable seasonal variation in mercury levels of Canadian pheasants. In early summer, shortly after mercury-treated seeds were available for ingestion by the birds, muscle residues averaged 0.45 ppm; but by late September these had declined to less than 0.1 ppm, suggesting a relatively short retention of mercury in pheasants.

LEAD

Lead residues were found in varying amounts in all of the 50 survey samples analyzed (Table 1). Whole body, wet-weight residues ranged from 0.12 ppm at a rural site near Spokane, Wash., to 6.6 ppm in birds collected from Quincy, Mass., near Boston. As expected, birds from urban sites had the highest residue levels, reflecting greater exposure to both automotive and industrial contamination. A comparison of sites where birds were collected both in 1970 and 1971 indicates that the 1971 figures are slightly lower in general. Crosschecks run by the two laboratories involved—Wisconsin Alumni Research Foundation in 1970 (1) and Denver Wildlife Research Center in 1971—suggest that the difference is probably due to analytical techniques and not to a reduced environmental burden of lead.

CADMIUM

Starlings were analyzed for cadmium residues for the first time in 1971; therefore, no comparative data are available. Residue levels ranged from <0.05 ppm (limit of sensitivity) to 0.24 ppm, with 14 of the 50 samples exceeding 0.05 ppm (Table 1). While the levels reported here are low, it is known that a certain amount of environmental cadmium is available to starlings in some of the food they consume. For example, levels reported by Schroeder *et al.* (8) in various grains ranged from 0.1 to 0.67 ppm.

Previous studies of cadmium levels in wildlife reported levels in specific body organs rather than in the whole birds (8,9). Since kidneys and liver concentrate body cadmium, residue levels reported in earlier studies were higher than those reported here. Examples of levels reported for animal kidneys are: deer 2.8-13.7 ppm, coyote 0.36 ppm, and starling 1.0 ppm. Reported liver residue levels are: ruffed grouse 0.88 ppm, squirrel 0.73 ppm, and domestic dog 0.59 ppm. Residue levels reported in seafood (including fish, crustaceans, and shellfish) ranged from .08 to 3.66 ppm. Mollusks appear to be particularly efficient at concentrating cadmium as both fresh and frozen oysters were reported to contain levels exceeding 3.0 ppm (8,9).

ARSENIC

As with cadmium, arsenic was included in the analyses for the first time in 1971. Arsenic residue levels generally were low, except for the sample from Michigan which

contained 0.21 ppm (Table 1). Remaining samples had residues of 0.04 ppm or less, with no residues detected in eight of these samples. Arsenic residues in urban soil samples around the country have been reported as high as 74.5 ppm (10), but apparently this arsenic either is unavailable to starlings or is ingested in a form which is not retained in the body of this species. It is known that the pentavalent form of arsenic is eliminated from human beings by excretion as rapidly as it is ingested. Trivalent arsenicals accumulate in human tissue, but there is very little contamination of the environment by this type (11).

Acknowledgment

Collections were made by field personnel of the Wildlife Services Division, Bureau of Sport Fisheries and Wildlife. Regional Pesticide Specialists of the Division of Wildlife Services were responsible for coordinating and reporting collections and assuring that samples were received by the contracting laboratory in proper condition. The Regional Pesticide Specialists at collection time were:

James B. Elder	Minneapolis, Minn.
Donald W. Hawthorne	Atlanta, Ga.
Robert H. Hillen	Albuquerque, N. Mex.
David J. Lenhart	Portland, Oreg.
John W. Peterson	Boston, Mass.

See Appendix for chemical names of compounds discussed in this paper.

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PESTICIDES IN WATER

*Pesticides in Selected Western Streams—1968-71*¹

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ABSTRACT

This paper presents data from the U.S. Geological Survey program for monitoring pesticides in the streams of the Western United States for the period October 1968 to September 1971. Data for the previous 3 years of the study were published in earlier issues of the Pesticides Monitoring Journal (3, 8).

Compounds determined include the common chlorinated insecticides and herbicides. Heptachlor and its epoxide were not detected during the 3-year period, and aldrin was found only once. DDT was the most frequently occurring insecticide, and 2,4,5-T the most common herbicide. The amounts observed were small; the maximum concentration of an insecticide was 0.46 µg/liter for DDT, and of an herbicide 0.99 µg/liter for 2,4-D. Concentrations were highest in water samples containing appreciable amounts of suspended sediments. Graphs are included to show insecticide and herbicide occurrences for the 4-year period (October 1967-September 1971) during which all 20 monitoring stations have been in operation.

Beginning in July 1970, the phosphorothioate insecticides—parathion, methyl parathion, malathion, and diazinon—were determined monthly on all samples. Malathion was not found during this period. Polychlorinated biphenyl (PCB's) compounds which were monitored for beginning in October 1969 were also detected at two stations.

Introduction

Data from the U.S. Geological Survey's pesticide monitoring program for the Western United States have been previously reported in this Journal (3,8). This paper presents data collected from October 1968 through September 1971 for the same 20 stations. The pesticides monitored include the chlorinated insecticides and her-

bicides previously reported, as well as chlordane and toxaphene. From January to June 1970, samples were analyzed quarterly for the phosphorus insecticides—methyl parathion, parathion, and diazinon. Beginning in July 1970, the samples were analyzed monthly for these compounds and also for malathion. Beginning in October 1969, the samples were analyzed routinely for polychlorinated biphenyl (PCB's) compounds.

Data Collection Sites

Each of the 20 sampling stations is a designated or operated U.S. Geological Survey irrigation-network location where inorganic water-quality and stream-discharge data are available. These stations are listed in Table 1 and their locations are shown in Fig. 1. Historical hydrologic and inorganic data for all 20 stations are available from U.S. Geological Survey offices in the respective States.

Sampling Procedures

Samples were collected in 1-quart Boston round glass bottles sealed with a Teflon-lined screw cap. These narrow-mouth bottles made possible the collection of depth-integrated samples. Two bottles were collected at each station; the contents of one was used for insecticide analysis and the other for herbicide analysis.

A duo-pak container, consisting of a form-fitting expanded polystyrene case placed in a corrugated cardboard carton, was used for mailing the samples and for protecting the bottles from frost, light, and shock. The package is compact and light weight, and very little breakage occurred. The samples were transported by

¹Water Resources Division, U.S. Geological Survey, U.S. Department of the Interior, Austin, Tex. 78701. (Publication authorized by the Director, U.S. Geological Survey.)

TABLE 1.—*Pesticide-monitoring stations in Western United States*

GEOLOGICAL SURVEY STATION NUMBER	STREAM AND LOCATION	PESTICIDE ANALYSIS STARTED (DATE)
6-2145	Yellowstone River near Billings, Mont.	10-11-67
6-4760	James River at Huron, S. Dak.	10-31-67
6-7660	Platte River at Brady, Nebr.	10-18-67
6-8070	Missouri River at Nebraska City, Nebr.	10-18-65
7-1305	Arkansas River below John Martin Reservoir, Colo.	10-16-65
7-2450	Canadian River near Whitefield, Okla.	12-13-67
7-2505	Arkansas River at Van Buren, Ark.	10-21-65
8-1140	Brazos River at Richmond, Tex.	2-01-66
8-1620	Colorado River at Wharton, Tex.	10-22-65
8-3965	Pecos River near Artesia, N. Mex.	11-02-67
8-4692	Rio Grande below Anzalduas Dam, Tex.	10-15-65
9-3150	Green River at Green River, Utah	11-22-67
9-5180	Gila River below Gillespie Dam, Ariz.	11-21-67
9-5255	Colorado River (Yuma Main Canal) below Colorado River Siphon, at Yuma, Ariz.	10-12-65
10-3350	Humboldt River near Rye Patch, Nev.	10-17-67
11-4070	Feather River near Oroville, Calif.	10-31-67
11-4255	Sacramento River at Verona, Calif.	11-29-65
12-5105	Yakima River at Kiona, Wash.	10-11-65
13-1545	Snake River at King Hill, Idaho	10-17-65
14-1057	Columbia River at The Dalles, Oreg.	10-27-65

airmail because herbicides, particularly 2,4-D, can decompose quite rapidly (4). The average time from sampling to receipt of the sample at the laboratory was 3 days.

Analytical Procedures

Analytical procedures were essentially the same as previously reported (8). Upon receipt of the sample, one bottle was designated for insecticide analysis and the other for herbicide analysis. The herbicide sample was acidified, and the bottles were then refrigerated until extraction was begun. No attempt was made to separate suspended sediment from the water for a separate analysis.

Insecticide samples were analyzed, together with a reagent blank, by the U.S. Geological Survey method described by Goerlitz and Brown (6) and summarized below. The entire sample (800-900 ml) was extracted with redistilled hexane and the extracts dried over anhydrous sodium sulfate before concentration to 2 ml in a Kuderna-Danish concentrator. From January to July 1970, samples were checked quarterly for several phosphorus pesticides using electron-capture detectors. Beginning in July 1970, a Tracor flame photometric detector was used in the phosphorus mode (526 nm filter) to screen the extracts prior to cleanup. The flame-

photometric detector was mounted on a MicroTek 220 equipped with two $\frac{1}{4}$ " $\times$ 6' pyrex columns packed with 3% OV-1 and 5% OV-210 as described by Bowman and Beroza (2).

After analysis for the phosphorothioate compounds, alumina cleanup, as described by Law and Goerlitz (7), was used before electron capture analysis. Occasional sulfur interference was eliminated by adding metallic mercury (5). Chlorinated insecticides were analyzed on both a Varian Model 200 dual-column chromatograph and a MicroTek Model 160. The columns used in the Varian instrument were $\frac{1}{8}$ " $\times$ 5' pyrex. One was packed with 3% OV-101 and the other with a mixed column made up of equal weights of 3% OV-1 and 5% OV-210 both on 100/120 mesh Gas Chrom Q. The $\frac{1}{4}$ " $\times$ 5' pyrex column in the MicroTek instrument was packed with the mixed OV-1/OV-210 phase.

Any pesticides suspected on one column were confirmed on the other column prior to reporting. When PCB compounds were suspected, they were verified by their elution pattern on an alumina cleanup column and the peak ratios were compared with a standard of the suspected compound to determine the presence or absence of the DDT family. After January 1971, the silicic acid cleanup method of Armour and Burke (1) was used on each sample suspected of containing PCB's.

Recovery data, as determined by extracting distilled water to which acetone solutions of insecticides had been added, ranged from 85 to 110%. No recovery corrections were made because most of the values reported were slightly above the lower detection limits of 0.005 μ g/liter for most of the chlorinated compounds; chlordane was detectable to about 0.1 μ g/liter, and toxaphene to 0.5 to 1.0 μ g/liter. The phosphorus compounds could be determined to 0.01 μ g/liter.

Herbicide samples were analyzed in sets of eight, along with a reagent blank, according to the method described by Goerlitz and Brown (6). Each acidified sample (800-900 ml) was extracted with redistilled ethyl ether. The extract was saponified using potassium hydroxide, and this basic solution was subjected to a cleanup extraction using ether to remove organic contamination which may have been present in the original sample. After the cleanup extraction, the solution was acidified and the phenoxy acid herbicides were extracted with ether. This extract was allowed to dry over acid sodium sulfate, the ether evaporated, and the herbicides esterified with boron trifluoride-methanol reagent.

After esterification, the samples were passed through a Florisil cleanup column, and the herbicide methyl esters eluted with benzene to a final volume of 2.0 ml. Herbi-

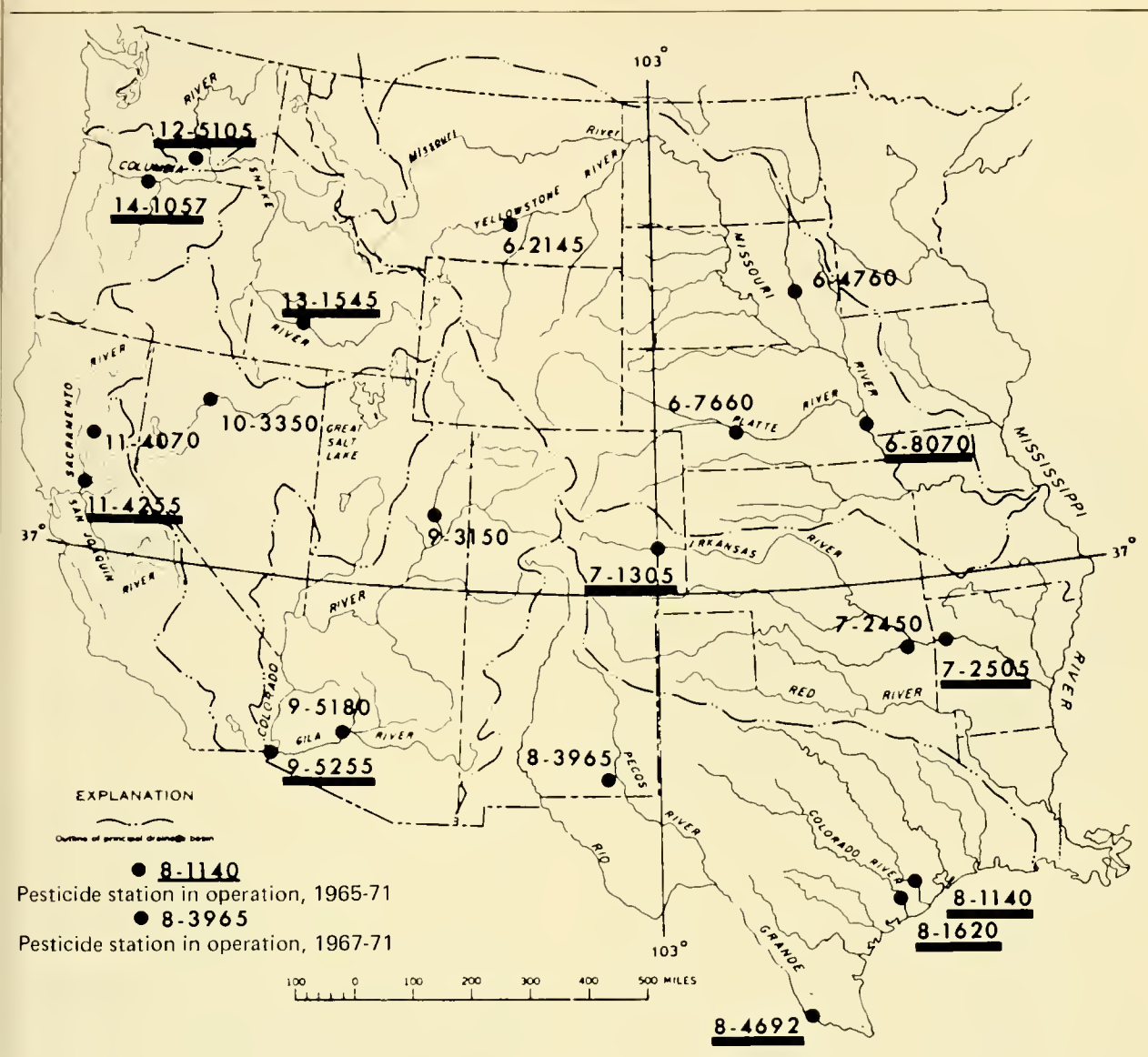


FIGURE 1.—Pesticide-monitoring stations in Western United States

cide methyl esters were analyzed on a dual-column Varian Model 200 chromatograph. One column was packed with 9% DC-200 and 1% Carbowax 20M on 60/80 mesh Gas Chrom Q and the other column was packed with 10% QF-1 and 0.5% Carbowax 20M on 60/80 mesh Chromosorb W.

Herbicide concentrations could be routinely determined as low as 0.005 µg/liter in most waters, with the exception of 2,4-D which was detectable to a level of 0.02 µg/liter. Spiked samples were used to check the entire extraction and cleanup procedures and recovery ranged from 85 to 110%. No corrections for recovery were made.

Discussion of Results

Results of analyses of water samples for the 3-year period—October 1968 to September 1971—are given in Table 2; the number of occurrences at each station of pesticides analyzed for during this entire period are given in Table 3. Heptachlor and its epoxide were not detected and aldrin was found only once during the 3-year period.

Samples from Yellowstone River near Billings, Mont., contained no insecticides. Six other streams were positive only once for the chlorinated insecticides, and five of these six streams showed no phosphorus compounds

during the final year. Pesticide concentrations were never in excess of the permissible limits established for public water supplies by the National Technical Advisory Committee to the Secretary of the Interior (9), although in several instances concentrations were measured that were above the environmental levels of 0.05 $\mu\text{g}/\text{liter}$ recommended for marine or estuarine waters.

The highest concentration of insecticide observed was 0.46 $\mu\text{g}/\text{liter}$ DDT in the November 1968 sample from Pecos River near Artesia, N. Mex., the allowable limit for DDT in public drinking-water supplies is 42 $\mu\text{g}/\text{liter}$.

Every station had occurrences of herbicides, but Green River at Green River, Utah, had only one occurrence. The highest concentration of herbicide found was 0.99 $\mu\text{g}/\text{liter}$ of 2,4-D in the sample from Feather River near Oroville, Calif., in December 1969. The established criteria permit 100 $\mu\text{g}/\text{liter}$ of the three herbicides combined in public drinking water supplies.

The total occurrences of chlorinated insecticides with the occurrence of DDT and its metabolites, DDD and DDE, for the period October 1967 to September 1971 are compared in Fig. 2. The data show that total occurrences of insecticides have declined since 1968 (Fig. 2). Occurrences of the DDT family of insecticides also decreased, and the ratio between the DDT family and all insecticides declined from 82% in 1968 to 65% in 1971.

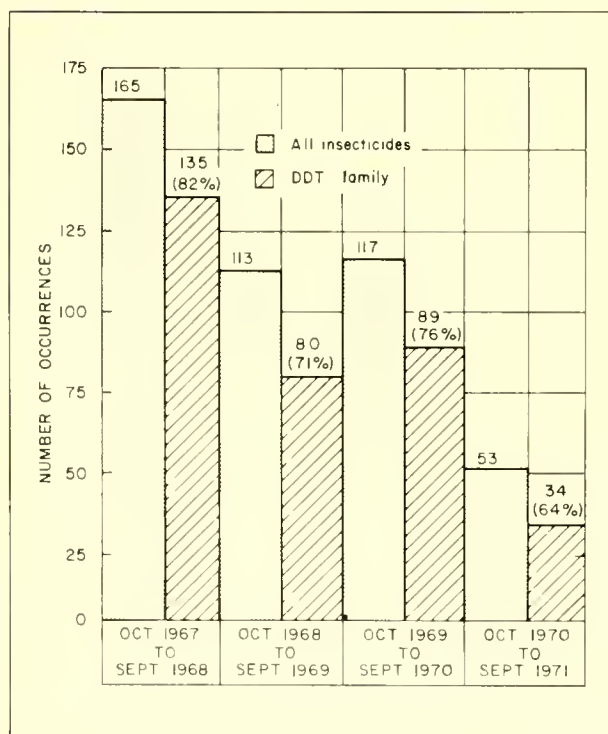


FIGURE 2.—Occurrences of chlorinated insecticides by year, October 1967 to September 1971

It should be noted that 36% of all insecticide occurrences and 33% of the occurrences of the DDT family insecticides were at one site: Gila River at Gillespie Dam, Ariz.

The occurrences of the three phenoxy acid herbicides for all 20 stations by year for the period October 1967 to September 1971 are shown in Fig. 3. Total occurrences reached a peak of 106 during 1968-69 and declined sharply to 54 during 1970-71. This decrease of about 50% was due to reduced occurrences of 2,4-D and 2,4,5-T.

Silvex occurrences did not change significantly over the 4-year period, and of the total silvex occurrences, 64% were detected at one station—Humboldt River near Rye Patch, Nev. Of the total 2,4,5-T occurrences, 47% were detected at two stations—Arkansas River at Van Buren, Ark., and Canadian River near Whitefield, Okla. The Canadian River is a tributary of the Arkansas River approximately 88 river miles upstream from the Van Buren site.

See Appendix for chemical names of compounds discussed in this paper.

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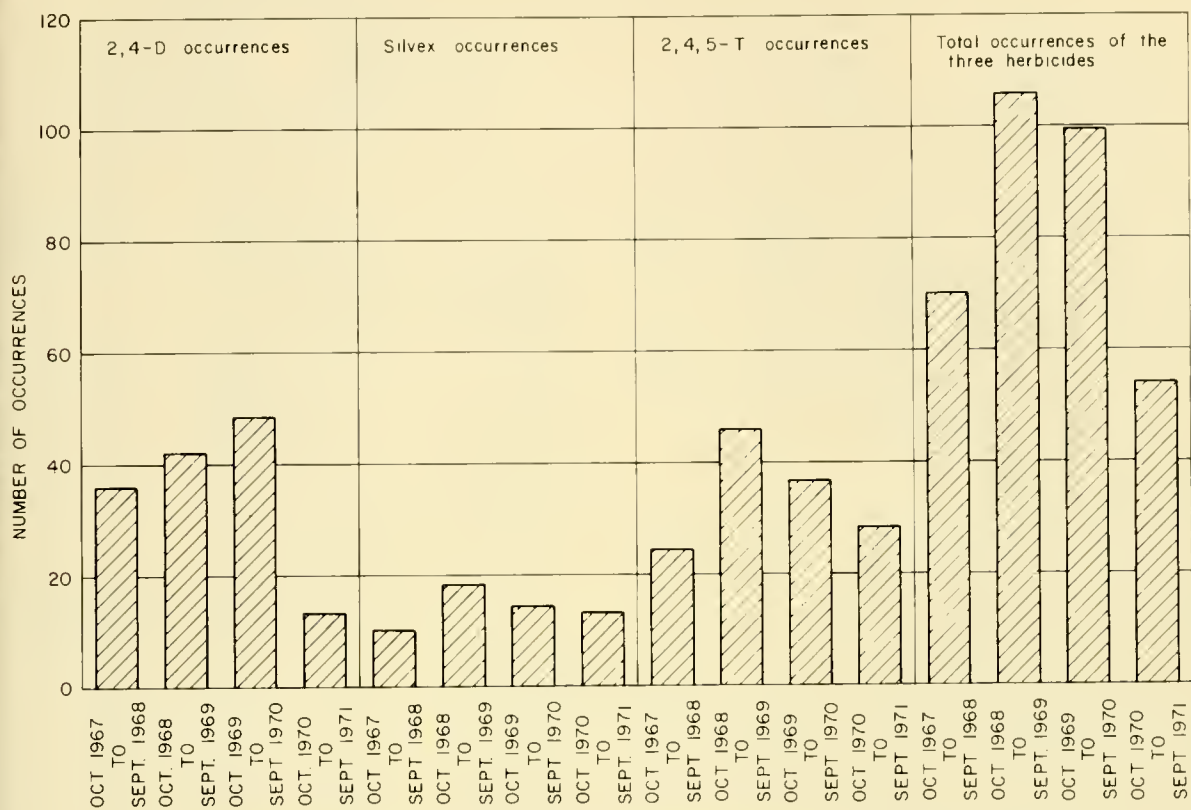


FIGURE 3.—Occurrences of herbicides by year, October 1967 to September 1971

TABLE 2.—Pesticide content of selected streams in Western United States

October 1968-September 1971, samples analyzed for aldrin, DDD, DDE, DDT, dieldrin, endosulfan, endrin, heptachlor, h. epoxide, lindane, chlordane, toxaphene, 2,4-D, silvex, and 2,4,5-T
beginning October 1969, samples analyzed for PCB's
January 1970-June 1970, samples analyzed *quarterly* for methyl parathion, parathion, and diazinon
beginning July 1970, samples analyzed *monthly* for methyl parathion, parathion, diazinon, as well as malathion

DATE	TIME (24 HOUR)	INSTANTANEOUS DISCHARGE (CFS)	RESIDUES IN µG/LITER	DATE	TIME (24 HOUR)	INSTANTANEOUS DISCHARGE (CFS)	RESIDUES IN µG/LITER
USGS NO. 6-8070 MISSOURI RIVER AT NEBRASKA CITY, NEBR.				USGS NO. 6-8070 MISSOURI RIVER—Continued			
0/16/68	1410	36100	.13 (2,4-D)	12/30/69	1300	¹ 20000	.05 (2,4-D)
1/13/68	1250	37400		01/26/70	1100	¹ 19000	.01 (DDT); .01 (Lindane); .05 (2,4-D)
2/11/68	1105	21000		02/24/70 ²	1100	36600	.03 (2,4-D); .01 (2,4,5-T)
1/20/69	1100	23000		03/12/70 ²	1300	33600	
2/12/69	1015	24000		04/08/70 ²	1400	43900	.06 (2,4-D)
3/26/69	1300	55600	.01 (DDT); .02 (Dieldrin)	05/22/70 ²	1105	37900	.01 (Dieldrin)
4/11/69	1430	99000		06/09/70 ²	1130	40000	.06 (2,4-D); .01 (2,4,5-T)
5/14/69	1300	50600		07/24/70 ²	1100	42400	.03 (2,4-D)
6/20/69	1300	42100		08/28/70 ³	1200	46100	
7/31/69	1120	48900		09/17/70 ³	1100	47200	.01 (DDD); .01 (DDT)
9/30/69	1110	55600	.01 (DDD); .01 (DDT); .01 (Dieldrin)	10/27/70 ³	1430	50000	
0/21/69	1000	50000		11/16/70 ³	1300	49200	
1/28/69	1030	39400		12/22/70 ³	1600	21400	

TABLE 2.—Pesticide content of selected streams in Western United States—Continued

DATE	TIME (24 HOUR)	INSTAN- TANEOUS Dis- CHARGE (CFS)	RESIDUES IN μ G/LITER
USGS NO. 6-8070 MISSOURI RIVER—Continued			
01/22/71 ^a	1130	20000	.01 (Lindane); .04 (2,4-D)
02/11/71 ^a	1430	20500	
03/16/71 ^a	1315	54500	.01 (DDT); .01 (Dieldrin)
04/08/71 ^a	1030	47800	
06/29/71 ^a	1100	58000	.05 (Diazinon)
07/16/71 ^a	0930	57300	.01 (Dieldrin); .10 (2,4,5-T)
08/05/71 ^a	1655	52000	.07 (2,4-D); .01 (Silvex)
09/14/71 ^a	1130	49200	

USGS NO. 6-2145 YELLOWSTONE RIVER NEAR BILLINGS, MONT.			
10/07/68	1350	6060	.05 (2,4-D); .01 (2,4,5-T)
11/06/68	1525	5290	
12/04/68	1600	3830	
01/10/69	1430	2970	
02/11/69	1400	3180	
03/10/69	1045	2860	.42 (2,4-D)
04/09/69	1355	5680	
05/05/69	1400	6250	
06/12/69	1030	24900	
07/15/69	1030	15600	
08/05/69 ^a	1045	6160	
09/11/69	1100	3140	
10/14/69	1100	4220	
11/11/69	1400	3940	
12/08/69	1345	2910	
01/07/70	1430	1330	
02/09/70	1345	3050	
03/10/70 ^a	1315	3020	.04 (2,4-D)
04/13/70	1115	3610	
05/04/70 ^a	1230	4340	
06/03/70	1130	23400	
07/14/70 ^a	1045	17800	
08/03/70 ^a	1330	8880	
09/01/70 ^a	1115	3920	
10/06/70 ^a	1500	4960	
11/02/70 ^a	1345	4180	
11/30/70 ^a	1200	4160	
01/21/71 ^a	1545	4800	
02/09/71 ^a	1515	3040	
03/02/71 ^a	1415	2980	
04/05/71 ^a	1345	3300	
05/10/71 ^a	1300	13400	
06/08/71 ^a	1200	24600	
07/08/71 ^a	1200	27400	
08/10/71 ^a	1300	8990	
09/02/71 ^a	1200	7500	

USGS NO. 6-4760 JAMES RIVER AT HURON, S. DAK.			
10/31/68	0820	0	.16 (2,4-D)
11/27/68	1030	75	.16 (2,4-D)
12/27/68	1515	68	.43 (2,4-D); .17 (2,4,5-T)
01/27/69	1330	27	
02/25/69	0900	20	
03/18/69	1520	52	.01 (Lindane)
04/25/69	1025	5000	.15 (2,4-D)
05/22/69	1335	2200	.14 (2,4-D)
06/16/69	1130	1080	.01 Lindane); .13 (2,4-D); .01 (2,4,5-T)
07/28/69	1330	960	.11 (2,4-D)
08/18/69	1440	674	
09/15/69	1015	243	.04 (2,4-D)
10/14/69	1530	56	.26 (2,4-D)

DATE	TIME (24 HOUR)	INSTAN- TANEOUS Dis- CHARGE (CFS)	RESIDUES IN μ G/LITER
USGS NO. 6-4760 JAMES RIVER—Continued			
11/12/69	0930	174	.03 (2,4-D)
12/02/69	1045	93	.07 (2,4-D)
01/09/70	1200	290	.05 (2,4-D)
02/18/70	1310	40	
03/12/70 ^a	1410	685	.19 (2,4-D)
04/09/70	1320	133	.06 (2,4-D)
05/26/70 ^a	1420	357	.05 (2,4-D)
06/24/70	1530	243	.39 (2,4-D)
07/16/70 ^a	1415	41	.38 (2,4-D)
08/12/70 ^a	1500	0	.14 (2,4-D)
09/17/70 ^a	0800	0	.11 (2,4-D)
10/13/70 ^a	1445	0	.04 (2,4-D)
11/17/70 ^a	0800	0	.40 (2,4,5-T)
12/30/70 ^a	1530	0	
03/01/71 ^a	1545	2.2	
03/16/71 ^a	1400	411	.01 (Lindane)
09/30/71 ^a	1605	2.0	

USGS NO. 6-7660 PLATTE RIVER AT BRADY, NEBR.			
10/04/68	1405	127	.21 (2,4-D)
11/03/68	1110	125	
12/02/68	1345	133	
01/07/69	1550	184	.09 (2,4-D); .02 (2,4,5-T)
02/06/69	1400	138	
03/04/69	1510	128	
04/01/69	1325	188	.03 (Silvex)
05/06/69	1315	134	
06/02/69 ^a	1415	123	
07/10/69	1550	172	
08/15/69	1105	1160	.06 (2,4-D)
09/26/69	1445	113	
10/23/69	1530	152	
11/20/69	1330	211	
12/12/69	1440	176	
01/26/70	1500	674	
02/20/70	1000	322	.01 (DDT)
03/13/70 ^a	1505	246	
04/17/70	1500	73	
05/14/70 ^a	1515	1160	
06/18/70	1130	1930	
07/20/70 ^a	1630	1280	
08/17/70 ^a	1430	585	
09/21/70 ^a	1445	103	
10/19/70 ^a	1520	115	
11/17/70 ^a	1350	131	
12/16/70 ^a	1530	133	
01/20/71 ^a	1530	362	
02/26/71 ^a	1450	393	
03/17/71 ^a	1415	196	
04/15/71 ^a	1500	251	
06/18/71 ^a	1515	9990	
07/22/71 ^a	1150	1860	
08/10/71 ^a	1330	1200	
09/23/71 ^a	1430	583	

USGS NO. 7-1305 ARKANSAS RIVER BELOW JOHN MARTIN RESERVOIR, COLO.			
10/24/68	1905	106	.03 (Silvex)
11/20/68	1500	26	
12/26/68	1345	2.0	
01/08/69	0930	1.7	.02 (DDD); .02 (2,4,5-T)
02/04/69	1410	2.3	
03/10/69	0910	2.6	

TABLE 2.—Pesticide content of selected streams in Western United States—Continued

DATE	TIME (24 HOUR)	INSTAN- TANEOUS DIS- CHARGE (CFS)	RESIDUES IN μ G/LITER	DATE	TIME (24 HOUR)	INSTAN- TANEOUS DIS- CHARGE (CFS)	RESIDUES IN μ G/LITER
USGS NO. 7-1305 ARKANSAS RIVER—Continued				USGS NO. 7-2505 ARKANSAS RIVER—Continued			
04/01/69 ⁴	0830	1.8		12/16/70 ³	0830	3830	
06/03/69	1600	485	.14 (2,4-D)	01/13/71 ³	0830	24900	.03 (2,4,5-T)
07/07/69	1030	994	.01 (Lindane)	02/11/71 ³	1300	16000	
08/19/69	0905	246	.02 (DDT); .02 (Dieldrin)	03/18/71 ³	1330	17400	
09/09/69	1330	61	.01 (DDD); .02 (DDE); .05 (DDT); .01 (Dieldrin); .04 (Lindane)	04/14/71 ³	0900	8320	.01 (2,4,5-T); .02 (Diazinon)
10/06/69	0915	75	.01 (Dieldrin)	05/12/71 ³	0830	12400	.01 (2,4,5-T)
11/03/69	1555	5.2		06/16/71 ³	0845	36700	.01 (2,4,5-T)
12/10/69	1240	3.0		07/23/71 ³	1000	13900	.01 (2,4,5-T)
01/06/70	1540	3.0		08/18/71 ³	0830	9450	.02 (2,4,5-T)
				10/01/71 ³	0900	15900	.03 (2,4,5-T)
02/05/70	1025	3.0		USGS NO. 7-2450 CANADIAN RIVER NEAR WHITEFIELD, OKLA.			
03/09/70 ²	0900	2.8		10/22/68	0715	800	.04 (Silvex); .05 (2,4,5-T)
04/07/70	1415	3.3	.02 (Lindane)	11/19/68	0750	410	.15 (2,4-D); .05 (2,4,5-T)
05/05/70 ²	1020	840		12/19/68	0815	885	.03 (2,4,5-T)
05/14/70 ²	1250	1080	.01 (DDT); .03 (2,4-D); .02 (2,4,5-T)	01/22/69	1030	506	.02 (DDD); .15 (2,4-D); .09 (2,4,5-T)
06/02/70	1300	595		02/27/69	1700	24100	.04 (2,4,5-T)
07/07/70 ²	1515	509	.01 (DDD); .01 (DDT); .01 (Dieldrin)	03/25/69	1500	13200	.04 (2,4,5-T)
08/04/70 ²	1515	348		04/30/69	0715	14700	.05 (2,4,5-T)
09/10/70 ³	0950	431		05/15/69	0750	5600	.04 (2,4,5-T)
10/06/70 ³	0835	393	.01 (Dieldrin)	06/19/69	0710	634	.04 (2,4,5-T)
11/03/70 ³	1000	116		07/25/69	1330	398	.03 (2,4,5-T)
12/02/70 ³	1100	96		08/27/69	1420	793	.01 (DDT); .03 (2,4,5-T)
01/12/71 ³	1615	3.3		09/25/69 ⁴	1400	156	
02/02/71 ³	1040	3.3		10/30/69	1425	964	.02 (2,4,5-T)
03/02/71 ³	1020	3.3		11/26/69	1430	12100	.03 (2,4,5-T)
04/02/71 ³	1530	1090	.01 (2,4,5-T)	12/16/69	1300	7320	
05/11/71 ³	1430	73		02/26/70 ²	1100	370	.04 (2,4,5-T)
06/16/71 ³	1430	352		03/24/70 ²	1130	374	.01 (DDT); .02 (2,4,5-T)
07/07/71 ³	1615	369		04/21/70 ²	1130	426	.03 (2,4,5-T)
08/10/71 ³	1300	261	.01 (DDE)	05/27/70 ²	1300	1230	.02 (2,4,5-T)
09/09/71 ³	0930	59	.04 (2,4-D)	06/25/70	1200	2840	.03 (2,4,5-T)
USGS NO. 7-2505 ARKANSAS RIVER AT VAN BUREN, ARK. (Beginning December 1970, samples collected at Lock and Dam 13, below Fort Smith, Ark.)				07/29/70 ²	1045	410	.03 (2,4,5-T)
10/15/68	1035	9190	.01 (DDT)	08/27/70 ³	1330	1430	.03 (2,4,5-T)
11/14/68	1335	15400		09/29/70 ³	1100	68	.01 (2,4,5-T)
12/18/68	1135	31300	.03 (2,4,5-T)	11/30/70 ³	1100	3230	
01/07/69	—	48300	.08 (DDD); .08 (2,4-D); .05 (2,4,5-T)	12/22/70 ³	1400	8000	.02 (2,4,5-T)
02/10/69	1500	54500	.11 (2,4-D); .06 (2,4,5-T)	01/20/71 ³	1435	10600	.03 (2,4,5-T)
03/19/69	1130	23000		02/02/71 ³	1500	7530	.01 (Lindane); .02 (2,4,5-T)
04/10/69	1120	50400		03/17/71 ³	1430	6480	.01 (Lindane)
05/08/69	1020	77800	.02 (2,4,5-T)	04/20/71 ³	1400	6180	.01 (2,4,5-T); .01 (Diazinon)
06/10/69	1010	59700	.07 (2,4-D); .07 (2,4,5-T)	05/12/71 ³	1500	150	.01 (2,4,5-T)
07/16/69	0952	32300	.04 (2,4,5-T)	07/14/71 ³	1515	705	
08/14/69	0830	18600	.03 (2,4,5-T)	08/18/71 ³	1330	150	
09/17/69	1000	15800	.02 (2,4,5-T)	09/08/71 ³	1400	1500	.09 (DDT); .02 (2,4,5-T)
10/14/69	0830	57100	.01 (DDD); .02 (DDT); .01 (Lindane); .05 (2,4,5-T)	USGS NO. 8-1140 BRAZOS RIVER AT RICHMOND, TEX.			
11/20/69	1205	16800	.02 (2,4,5-T)	10/07/68	1330	3590	.01 (DDD); .01 (DDE); .01 (DDT); .02 (2,4,5-T)
12/16/69	1335	8900		11/26/68	1245	1920	.01 (DDE); .01 (DDT)
01/23/70	1355	14600	.01 (DDD); .09 (DDT)	12/23/68	1210	7400	.01 (DDE); .01 (DDT)
02/17/70	1405	6140	.05 (DDT); .01 (2,4,5-T)	01/28/69	1320	2600	
03/13/70 ²	0945	17600		03/13/69	1115	8760	.01 (DDD); .02 (DDE); .04 (DDT); .02 (2,4,5-T)
04/17/70	1245	53100	.01 (DDT); .01 (Dieldrin); .03 (2,4-D); .02 (2,4,5-T)	04/18/69	0925	33700	.01 (DDD); .05 (DDE); .04 (DDT); .18 (2,4-D); .04 (2,4,5-T)
06/17/70	1425	43600	.06 (2,4-D); .06 (2,4,5-T)	06/06/69	1415	12200	.05 (2,4-D); .02 (2,4,5-T)
07/17/70 ²	1500	14600	.01 (DDE); .05 (DDT); .02 (2,4,5-T)	06/13/69	1320	6320	.06 (2,4-D); .02 (2,4,5-T)
08/17/70 ³	0905	1570	.06 (DDT); .02 (2,4,5-T); .07 (Diazinon)	07/22/69	1115	2200	
09/18/70 ³	1010	16000	.02 (2,4,5-T)	08/19/69	1345	1520	
10/13/70 ³	1335	44400	.05 (DDT); .06 (2,4,5-T)	10/09/69	1400	946	
11/18/70 ³	1000	17900	.01 (DDT)	10/20/69	1115	1370	

TABLE 2.—Pesticide content of selected streams in Western United States—Continued

DATE	TIME (24 HOUR)	INSTAN- TANEOUS DIS- CHARGE (CFS)	RESIDUES IN µG/LITER	DATE	TIME (24 HOUR)	INSTAN- TANEOUS DIS- CHARGE (CFS)	RESIDUES IN µG/LITER
USGS NO. 8-1140 BRAZOS RIVER—Continued				USGS NO. 8-4692 RIO GRANDE BELOW ANZALDUAS DAM, TEX.			
10/27/69	1110	1310	.01 (2,4,5-T)	10/15/68	0745	540	.01 (DDE)
12/04/69	1110	1510		11/20/68	1010	2240	.02 (Silvex)
02/19/70	1230	4610	.01 (2,4,5-T)	12/26/68	1240	1320	
03/19/70 ^a	1605	21600	.03 (DDD); .04 (DDE); .08 (DDT); .04 (2,4-D); .02 (2,4,5-T)	01/16/69	0830	3090	
04/29/70 ^a	1640	11100	.01 (DDD); .01 (DDE); .01 (DDT); .01 (2,4,5-T)	02/17/69	1020	650	
05/18/70 ^a	0950	7070	.06 (2,4-D); .01 (2,4,5-T)	03/13/69	0930	381	
06/01/70	1200	16400	.03 (DDD); .04 (DDE); .06 (DDT); .01 (Lindane); .07 (2,4-D); .01 (2,4,5-T)	04/14/69	1135	1400	
				05/15/69	0805	501	.01 (DDE)
				06/19/69	0740	4310	
				07/16/69	0735	1910	.02 (DDD); .01 (DDE); .01 (DDT); .01 (Lindane)
06/23/70	1415	3920					
07/16/70 ^a	1425	1370		08/27/69	0850	509	
08/18/70 ^a	1130	1150	.2 (PCB's)	09/18/69	0730	699	
08/19/70 ^{a,4}	1330	988		10/13/69	1210	800	
11/03/70 ^a	1315	4370	.01 (DDE); .02 (2,4,5-T); .3 (PCB's)	11/18/69	1310	1000	
12/17/70 ^a	1430	754	.02 (Dieldrin); .5 (PCB's)	12/17/69	0900	1240	
01/26/71 ^a	1510	910		01/19/70	1200	300	.02 (DDT)
02/17/71 ^{a,4}	1500	595	.1 (PCB's)	02/24/70 ^a	1100	330	
04/07/71 ^a	1330	510	.01 (2,4,5-T)	03/16/70 ^a	0900	1020	
06/15/71 ^a	1315	625	.06 (DDE); .39 (DDT)	04/07/70	0830	1770	
06/16/71 ^a	1315	940	.2 (PCB's)	05/11/70 ^a	0745	2610	.01 (DDE); .01 (2,4,5-T)
07/21/71 ^a	1600	575		06/18/70	0830	1590	
08/18/71 ^a	1100	1630		07/16/70 ^a	1135	100	.01 (DDD); .01 (DDE); .01 (DDT); .23 (Methly parathion; 1.0 (Parathion)
08/18/71 ^{a,4}	1445	1590	.01 (DDE); .01 (DDT); .01 (Dieldrin); .4 (PCB's)				
09/24/71 ^a	1355	1280		08/19/70 ^a	0900	1260	
				10/13/70 ^a	0800	125	
				11/16/70 ^a	1045	400	
USGS NO. 8-1620 COLORADO RIVER AT WHARTON, TEX.							
10/01/68	1400	579		12/18/70 ^a	1115	600	
12/19/68	1215	680	.02 (2,4,5-T)	01/15/71 ^a	1210	2200	
01/21/69	1440	845	.03 (DDD)	02/26/71 ^a	1105	1040	
03/05/69	1255	1430	.01 (Aldrin); .01 (DDT); .01 (Dieldrin)	03/17/71 ^a	1025	1100	
				04/16/71 ^a	1135	200	
04/23/69	0745	3870	.01 (DDE); .02 (DDT); .01 (2,4,5-T)				
				05/20/71 ^a	0945	2200	
06/04/69	0950	2050		06/17/71 ^a	0800	3000	
06/13/69	1045	1380	.04 (DDT); .03 (2,4,5-T)	07/19/71 ^a	0955	400	.01 (2,4,5-T)
07/09/69	0950	1150	.01 (2,4,5-T)	08/12/71 ^a	1020	10	
08/21/69	1745	900		10/15/71 ^a	1155	41200	.02 (DDE)
10/09/69	1200	395	.02 (DDT); .01 (Lindane)				
				USGS NO. 8-3965 PECOS RIVER NEAR ARTESIA, N. MEX.			
10/20/69	0930	2980		10/01/68	1340	3.8	
10/29/69	1610	2520	.01 (DDD)	11/01/68	0925	34	.02 (DDD); .01 (DDE); .46 (DDT); .01 (Lindane)
12/02/69	1520	2670					
01/14/70	1630	3280		12/02/68	1225	45	.15 (2,4-D)
02/19/70	1045	2020		01/03/69 ^a	1145	42	.10 (2,4-D); .05 (2,4,5-T)
03/19/70 ^a	1400	9160	.01 (DDD); .01 (DDE); .03 (DDT); .05 (2,4-D)	02/04/69	1225	40	
04/29/70 ^a	1335	4150	.01 (DDT)	03/03/69	1225	36	.02 (Chlordane)
05/18/70 ^a	1650	24200	.02 (DDD); .02 (DDE); .03 (DDT); .40 (2,4-D); .06 (2,4,5-T)	04/01/69	1230	787	
				05/01/69	1345	33	
06/23/70	1250	2240		06/02/69	1045	12	
07/16/70 ^a	1114	3360		07/01/69	1320	646	
08/18/70 ^a	1000	935		08/01/69 ^a	1045	6.4	
08/19/70 ^{a,4}	1125	990		09/02/69	1250	230	.02 (DDT)
09/11/70 ^a	1100	1190		10/01/69	1345	101	
10/27/70 ^a	1400	3760	.01 (2,4,5-T)	11/04/69	1410	157	.01 (Dieldrin); .02 (Lindane)
12/17/70 ^a	1225	445		12/01/69	1140	78	
01/28/71	1235	358		01/02/70	1345	74	.01 (Lindane)
02/17/71 ^a	1200	284	.04 (2,4,5-T)	02/02/70	1305	64	
04/07/71 ^a	1215	860		03/02/70 ^a	1310	52	.01 (Lindane); .08 (Methyl parathion)
06/03/71 ^a	0955	595		04/01/70	1240	9.6	
06/15/71 ^a	1205	885		05/01/70 ^a	1115	6.2	
06/16/71 ^a	1100	920		06/01/70	1345	693	
07/21/71 ^a	1235	691		07/01/70 ^{a,4}	1150	214	
				08/03/70 ^a	1430	703	
08/18/71 ^a	1300	639		09/01/70 ^a	1240	24	.01 (DDT)
08/18/71 ^a	1330	639					
09/21/71 ^a	1730	1770					

TABLE 2.—Pesticide content of selected streams in Western United States—Continued

DATE	TIME (24 HOUR)	INSTAN- TANEOUS DIS- CHARGE (CFS)	RESIDUES IN $\mu\text{G/LITER}$	DATE	TIME (24 HOUR)	INSTAN- TANEOUS DIS- CHARGE (CFS)	RESIDUES IN $\mu\text{G/LITER}$
USGS NO. 8-3965 PECOS RIVER—Continued				USGS NO. 9-3150 GREEN RIVER—Continued			
10/01/70 ^a	1400	91		05/12/69	1645	19600	
11/02/70 ^a	1315	54		06/10/69	1320	9550	
12/02/70 ^a	1230	50		07/03/69	1100	9690	
01/04/71 ^a	1430	45		08/14/69	1230	4590	
02/01/71 ^a	1245	37		09/17/69	1340	4210	
03/01/71 ^a	1245	38	.01 (Silvex)	10/08/69	1105	3080	
04/01/71 ^a	1215	6.2	.01 (Silvex); .04 (2,4,5-T)	11/13/69	1155	3800	
05/03/71 ^a	1145	57		12/11/69	1650	3540	
06/01/71 ^a	1330	39		01/08/70	1350	2740	
07/01/71 ^a	1230	.96		02/06/70	1520	2520	
08/02/71 ^a	1120	422	.01 (DDT)	03/09/70 ^a	1330	3400	.1 (PCB's)
09/01/71 ^a	0900	53	.04 (2,4-D)	04/09/70	1205	2970	
				05/13/70 ^a	1330	10900	.01 (DDT)
USGS NO. 9-5255 COLORADO RIVER (YUMA MAIN CANAL) AT YUMA, ARIZ. (Beginning October 1970, samples collected at Imperial Dam, 15 miles northeast of Yuma)				06/16/70	1400	18400	
10/01/68	0930	556		07/09/70 ^a	1315	9290	
11/05/68	1030	476		08/06/70 ^a	1245	3260	.15 (2,4,5-T)
12/03/68	1430	206		09/09/70 ^a	1115	3130	
01/07/69	0930	3.0	.14 (2,4-D); .03 (2,4,5-T)	10/06/70 ^a	1215	2900	
02/04/69	1000	365		11/11/70 ^a	1230	2560	
03/04/69	0915	411	.24 (2,4-D); .04 (Silvex); .07 (2,4,5-T)	12/08/70 ^a	1115	2580	
04/08/69	1315	492		01/20/71 ^a	1325	2560	
05/06/69	0920	422		02/09/71 ^{a,1}	1110	2860	
06/03/69 ¹	0940	639		03/09/71 ^a	1000	2600	
07/01/69	0930	610		04/12/71 ^a	1230	5810	
08/11/69	0825	547		05/11/71 ^a	1300	11400	
09/02/69	1345	467		06/08/71 ^a	1300	14800	
10/07/69	1030	625		07/06/71 ^a	1200	9060	
11/04/69	1115	274		08/10/71 ^{a,1}	1330	3380	
12/02/69	1100	75		09/08/71 ^a	1245	3610	
01/06/70	1100	274		USGS NO. 9-5180 GILA RIVER ABOVE DIVERSIONS AT GILLESPIE DAM, ARIZ. (Formerly published as USGS No. 9-5195 Gila River below Gillespie Dam, Ariz.; for this location, discharge reported is daily mean discharge)			
02/03/70	1115	373		10/16/68	1000	22	.03 (DDD); .08 (DDE); .02 (DDT); .03 (Dieldrin); .01 (Lindane)
03/03/70 ^a	0930	119		11/19/68	1200	21	.01 (DDD); .04 (DDE); .02 (DDT); .01 (Dieldrin); .01 (Lindane); .11 (Silvex)
04/07/70	1100	711		12/16/68	1245	19	.01 (DDD); .10 (DDE); .05 (DDT); .03 (Dieldrin); .07 (Lindane)
05/05/70 ^a	1030	637		01/15/69	1000	33	.02 (DDD); .04 (DDE); .01 (DDT); .01 (Dieldrin); .01 (Lindane); .06 (Silvex)
06/02/70	1045	586		02/17/69	1025	26	.03 (DDE); .01 (DDT); .01 (Dieldrin); .01 (Lindane)
07/07/70 ^a	0930	469		03/14/69	1315	29	.02 (DDE); .01 (Lindane)
08/04/70 ^a	1000	609		04/17/69	1240	28	.02 (DDE); .01 (Dieldrin); .02 (Lindane)
09/01/70 ^a	1330	400		05/20/69	1315	26	.01 (DDD); .02 (DDE)
10/27/70 ^a	1100	6870		06/18/69	1410	26	.01 (DDD); .06 (DDE); .02 (Dieldrin); .01 (Lindane)
11/10/70 ^a	1130	5360		07/16/69 ^a	1300	12	
12/01/70 ^a	1015	3710	.01 (DDT)	08/20/69	1100	7.9	.02 (DDD); .05 (DDE); .03 (DDT)
01/05/71 ^a	0820	6480		09/17/69	1120	20	.02 (DDE); .03 (DDT); .03 (Endrin); .01 (Lindane)
02/02/71 ^a	0930	6160		10/13/69	0915	14	.01 (DDD); .01 (DDE); .01 (DDT); .01 (Dieldrin); .01 (Endrin); .02 (Lindane); .01 (2,4,5-T)
03/02/71 ^a	1445	8770		11/21/69	1300	26	.01 (DDD); .03 (DDE); .01 (DDT); .02 (Lindane); .05 (2,4-D)
04/06/71 ^a	0915	11200		12/18/69	1205	7.2	.04 (DDD); .02 (DDE); .02 (DDT); .02 (Lindane); .03 (2,4-D)
05/04/71 ^a	1000	8830		01/22/70	1030	31	.02 (DDD); .04 (DDE); .02 (DDT); .01 (Lindane)
06/01/71 ^a	1030	9190		02/17/70	1215	23	.02 (DDD); .04 (DDE); .01 (DDT); .01 (Endrin); .02 (Lindane); .02 (Endosulfan)
07/06/71 ^a	0835	10900					
08/03/71 ^a	0826	10900					
09/07/71 ^a	0815	7800					
USGS NO. 9-3150 GREEN RIVER AT GREEN RIVER, UTAH							
10/14/68	1800	3620					
11/18/68	1000	3850	.01 (DDT)				
12/13/68	1245	4330					
01/15/69	1345	3880					
02/11/69	1320	5720					
03/14/69	1240	5750					
04/10/69	1045	11100					

TABLE 2.—Pesticide content of selected streams in Western United States—Continued

DATE	TIME (24 HOUR)	INSTAN- TANEOUS Dis- CHARGE (CFS)	RESIDUES IN µG/LITER
USGS NO. 9-5180 GILA RIVER ABOVE DIVERSIONS AT GILLESPIE DAM, ARIZ.—Continued			
04/01/70 ^{2,4}	1200	1.3	.02 (DDD); .05 (DDE); .01 (DDT); .01 (Lindane)
05/04/70 ²	1345	22	.02 (DDD); .03 (DDE); .01 (DDT)
06/03/70	1140	7.3	.01 (DDD); .04 (DDE); .01 (DDT)
07/02/70 ²	1530	4.0	.01 (DDD); .02 (DDE); .01 (Dieldrin)
07/30/70 ²	1500	5.4	.01 (DDD); .04 (DDE)
09/01/70 ²	1200	17	.02 (DDE); .04 (Methyl parathion); .04 (Parathion)
10/01/70 ²	1210	36	.01 (DDE); .01 (Lindane); .02 (Methyl parathion); .04 (Parathion)
11/02/70 ²	1230	38	.02 (DDE); .10 (Methyl parathion); .10 (Diazinon)
12/02/70 ²	1500	65	.01 (DDE); .06 (Lindane)
01/08/71 ²	1600	49	.05 (DDE); .02 (DDT); .01 (Dieldrin); .05 (Lindane)
02/01/71 ²	1030	26	.01 (DDE); .01 (Dieldrin); .01 (Lindane)
03/02/71 ²	1030	39	.01 (DDE); .01 (Dieldrin); .01 (Lindane)
04/06/71 ²	1245	35	.02 (DDD); .08 (DDE); .01 (Lindane)
05/07/71 ²	1030	28	.02 (DDE); .01 (Diazinon)
06/01/71 ²	1200	36	.02 (DDE); .04 (Diazinon)
07/01/71 ²	1130	10	.02 (DDE)
08/18/71 ²	1030	302	.01 (Silvex)
09/01/71 ²	1640	27	.03 (DDE); .05 (DDT)

USGS NO. 10-3350 HUMBOLDT RIVER NEAR
RYE PATCH, NEV.

10/01/68	0930	48	.14 (Silvex)
11/01/68	0845	0	.05 (Silvex)
12/02/68	0900	.6	.01 (Silvex)
12/31/68	1000	.6	.18 (2,4-D); .07 (2,4,5-T)
02/03/69	0915	.8	
03/03/69	0900	.9	.02 (Silvex)
04/01/69	1100	2.0	.02 (Silvex)
05/01/69	0915	602	.01 (DDT); .07 (Silvex)
06/02/69	0845	642	.10 (Silvex)
06/30/69	1030	320	.11 (Silvex)
08/01/69	0900	288	.08 (Silvex)
09/02/69	1200	180	.07 (Silvex)
10/01/69	1200	114	.06 (Silvex)
11/03/69	1030	137	.05 (Silvex)
12/01/69	0845	13	.03 (Silvex)
01/02/70	1300	16	.05 (Silvex)
02/02/70	0930	16	.05 (Silvex)
03/03/70 ²	0930	15	.05 (Silvex)
04/01/70	1030	142	.01 (Lindane); .05 (Silvex)
05/01/70 ²	1300	495	.06 (Silvex)
06/01/70	1100	396	.04 (Silvex)
07/01/70 ²	1000	310	.03 (Silvex)
08/31/70 ²	1315	195	.01 (Silvex)
10/01/70 ²	1130	105	.02 (Silvex)
11/03/70 ²	1015	49	.02 (Silvex)
12/01/70 ²	1100	16	.02 (Silvex)
12/31/70 ²	1030	13	.01 (Silvex)
02/01/71 ²	1200	68	.01 (Silvex)
03/01/71 ²	1200	333	.02 (Silvex)
04/01/71 ²	1100	523	.02 (Silvex)
05/03/71 ²	1130	867	.01 (Silvex)
06/01/71 ²	1100	1580	
07/02/71 ²	1200	1570	
09/01/71 ²	1100	238	

DATE	TIME (24 HOUR)	INSTAN- TANEOUS Dis- CHARGE (CFS)	RESIDUES IN µG/LITER
USGS NO. 11-4255 SACRAMENTO RIVER AT VERONA, CALIF.			
11/15/68	1100	12000	
12/12/68	1100	23200	
01/17/69	1145	59300	
03/05/69	1630	56700	
04/17/69	1110	37200	
06/05/69	0930	22600	
07/03/69	1000	11700	
08/19/69	0945	16600	.01 (DDT); .02 (2,4-D); .03 (2,4,5-T)
10/27/69	1540	12700	.04 (2,4-D); .01 (Silvex); .02 (2,4,5-T)
12/04/69	1400	12800	
12/30/69	1045	60300	.01 (2,4,5-T)
03/03/70 ²	1430	51300	.07 (2,4-D)
04/08/70	0915	14200	
05/21/70 ²	1035	11300	.02 (2,4-D); .01 (2,4,5-T); .10 (Methyl parathion); .16 (Parathion)
07/01/70 ²	1025	10700	.01 (Silvex)
08/11/70 ²	1135	11900	.01 (Silvex)
09/24/70 ²	0900	13900	
10/29/70 ²	0950	14000	
11/25/70 ²	0930	21700	
12/09/70 ²	0930	61600	
01/20/71 ²	1300	62800	
02/09/71 ^{2,4}	1445	30900	
02/24/71 ²	0930	18100	
04/07/71 ²	1100	38300	
05/10/71 ²	1005	27400	.13 (Methyl parathion); .04 (Parathion)
06/16/71 ²	0915	22700	
07/21/71 ²	0900	16500	
08/26/71 ²	0910	22400	
09/21/71 ²	0930	21600	

USGS NO. 11-4070 FEATHER RIVER NEAR OROVILLE, CALIF.

10/08/68	1330	393	.02 (DDT)
11/01/68	1645	393	
12/—/68	1430	382	.99 (2,4-D); .18 (2,4,5-T)
01/03/69	0800	420	.06 (2,4-D); .07 (Silvex); .03 (2,4,5-T)
02/04/69	0800	411	
03/04/69	0735	4930	
03/28/69	1700	1730	
05/01/69	0700	451	.06 (2,4,5-T)
06/05/69	1245	400	.01 (2,4,5-T)
07/02/69	1200	420	
07/30/69 ¹	1000	410	
09/04/69	0800	382	
09/30/69	1745	411	
10/30/69	1015	461	
11/26/69	0928	426	
12/31/69	1022	403	
01/29/70	1040	35200	
02/26/70 ²	0915	419	
03/27/70	0730	417	
04/29/70 ²	1500	420	
05/27/70 ²	1230	410	
06/24/70 ²	—	415	
08/03/70 ²	1500	392	
08/31/70 ²	1430	421	
09/28/70 ²	1730	410	
11/03/70 ²	1300	409	.02 (2,4,5-T)
12/01/70 ²	0815	409	
12/30/70 ²	1605	409	
01/29/71 ²	1130	405	
02/26/71 ²	1025	406	

TABLE 2.—Pesticide content of selected streams in Western United States—Continued

DATE	TIME (24 HOUR)	INSTAN- TANEOUS DIS- CHARGE (CFS)	RESIDUES IN μ G/LITER	DATE	TIME (24 HOUR)	INSTAN- TANEOUS DIS- CHARGE (CFS)	RESIDUES IN μ G/LITER
USGS NO. 11-4070 FEATHER RIVER NEAR OROVILLE, CALIF.—Continued				USGS NO. 13-1545 SNAKE RIVER AT KING HILL, IDAHO—Continued			
03/26/71 ¹	0830	433	.03 (Silvex)	04/18/69	1545	10700	
04/29/71 ¹	1300	409		05/23/69	1450	9860	.09 (2,4-D)
05/24/71 ¹	1500	411		06/23/69 ¹	0830	5330	
06/29/71 ¹	1730	413		07/14/69	1115	6190	.06 (2,4-D)
07/29/71 ¹	1300	404		08/11/69	1030	4950	.02 (DDT); .03 (2,4-D)
08/26/71 ¹	1400	411		09/08/69	1235	8210	.03 (DDT); .05 (2,4-D)
09/21/71 ¹	1300	414		10/06/69	1515	12200	.05 (DDT); .04 (2,4-D)
USGS NO. 12-5105 YAKIMA RIVER AT KIONA, WASH.				11/09/69	1430	12000	
11/25/68	1530	4070		12/15/69	1655	11700	.01 (DDD); .03 (DDT); .01 (Dieldrin); .02 (2,4-D)
12/16/68	1040	3480		01/13/70	1635	13000	.12 (DDT)
01/20/69	1545	3820		02/09/70	1155	10200	.01 (DDT)
02/25/69	1145	3230		03/15/70 ²	1345	7440	.03 (DDT)
03/26/69	0845	6150		04/20/70	1200	14900	.01 (DDT); .16 (Lindane)
04/09/69	0930	6960	.20 (2,4-D)	04/30/70 ²	0930	13700	
05/19/69	1530	8200	.01 (DDD); .01 (DDE); .04 (DDT); .24 (2,4-D)	05/11/70 ²	1450	17600	.01 (DDT)
06/27/69	1015	1720	.01 (DDE); .04 (DDT); .01 (Dieldrin); .21 (2,4-D)	06/08/70 ²	1340	10300	.04 (2,4-D)
07/30/69	0930	1300	.01 (DDD); .03 (DDT); .02 (Dieldrin); .18 (2,4-D)	07/11/70 ²	1550	7520	.01 (DDT); .06 (2,4-D)
08/21/69	1305	1550	.01 (DDD); .01 (DDE); .02 (DDT); .01 (Dieldrin); .09 (2,4-D)	08/09/70 ²	1515	9870	.04 (2,4-D)
09/09/69	1500	1740	.09 (2,4-D)	09/18/70 ²	1530	11100	.01 (DDT)
10/17/69	1510	3090	.01 (DDT); .04 (2,4-D)	10/05/70 ²	1045	11200	.06 (2,4-D)
11/25/69	1430	1940		11/20/70 ²	1725	13400	.01 (DDT)
12/30/69	1330	2120	.05 (2,4-D)	01/08/71 ¹	1030	14100	.01 (2,4,5-T)
01/27/70 ²	0955	5350	.04 (2,4-D)	01/28/71 ¹	1300	17100	
02/16/70 ²	1545	4360	.01 (Dieldrin); .04 (2,4-D)	02/27/71 ¹	1600	14600	
03/19/70 ²	1210	4390		03/27/71 ¹	1315	16600	
04/28/70 ²	1255	1790	.01 (DDT); .05 (2,4-D)	05/05/71 ¹	1345	25200	
05/25/70 ²	1630	4440	.01 (DDD); .01 (DDE); .02 (DDT); .42 (2,4-D); .01 (2,4,5-T)	05/25/71 ¹	1258	18500	.02 (2,4,5-T)
06/22/70	1055	2640	.02 (DDD); .01 (DDE); .02 (DDT); .01 (Dieldrin); .15 (2,4-D); .01 (2,4,5-T)	06/21/71 ¹	1305	14400	
07/20/70 ²	1155	1300	.01 (Dieldrin); .16 (2,4-D); .01 (2,4,5-T)	08/02/71 ¹	1800	7660	.02 (2,4-D)
08/24/70 ²	1420	1600	.01 (DDD); .01 (DDE); .18 (2,4-D)	08/30/71 ¹	1435	7630	
09/30/70	1415	1820		USGS NO. 14-1057 COLUMBIA RIVER AT THE DALLES, OREG.			
10/15/70 ²	1140	2330	.04 (2,4-D)	11/05/68	1030	144000	
11/24/70 ²	1150	1920		12/09/68	1000	122000	.26 (2,4-D); .07 (2,4,5-T)
12/29/70 ²	1015	1970		01/22/69	1430	230000	
01/22/71 ¹	1025	7600	.06 (2,4-D); .01 (2,4,5-T)	03/18/69	1430	207000	
02/03/71 ¹	1415	11200		05/07/69	1230	316000	
03/01/71 ¹	1435	4490		06/26/69	0830	292000	
04/05/71 ¹	1200	3690		08/06/69	1200	127000	
05/07/71 ¹	1640	10200	.01 (DDT)	09/16/69	1530	87000	.01 (2,4-D)
06/21/71 ¹	1150	5940	.01 (DDT); .07 (2,4-D)	10/21/69	1545	112000	
07/16/71 ¹	1115	2200	.01 (DDT); .27 (2,4-D)	12/02/69	1435	163000	
08/06/71 ¹	1635	1240	.02 (DDE); .01 (DDT); .01 (Dieldrin); .18 (2,4-D)	01/14/70	1130	127000	
09/23/71 ^{1,3,4}	1355	2380		03/03/70 ²	1610	197000	
USGS NO. 13-1545 SNAKE RIVER AT KING HILL, IDAHO				04/13/70	1415	168000	
10/01/68	1315	10700	.02 (DDT)	06/26/70 ²	1200	299000	.25 (2,4-D)
11/04/68	1115	10600		08/20/70 ²	1400	155000	
12/14/68	1100	12940		11/05/70 ²	1200	125000	
01/20/69	1100	16800	.04 (2,4,5-T)	12/17/70 ²	1300	106000	
03/07/69	1300	16000		01/21/71 ¹	1400	193000	
				03/09/71 ¹	0950	211000	.01 (DDT)
				04/14/71 ¹	1010	227000	
				06/09/71 ¹	1545	512000	
				07/15/71 ¹	0940	300000	
				09/01/71 ¹	1010	136000	

¹ Daily mean discharge.² Sample also analyzed for methyl parathion, parathion, and diazinon.³ Sample also analyzed for methyl parathion, parathion, diazinon, and malathion.⁴ Sample not obtained for 2,4-D, silvex, or 2,4,5-T analysis.⁵ Sample not obtained for organochlorine insecticide analysis.

TABLE 3.—Number of occurrences of pesticides, October 1968 to September 1971

STREAM AND LOCATION	NUMBER OF OCCURRENCES															
	ALDRIN	DDD	DDE	DDT	DIELDRAN	ENDRIN	HEPTACHLOR	HEPTACHLOR EPOXIDE	LINDANE	CHLORDANE	TOXAPHENE	ENDOSULFAN	2,4-D	SILVEX	2,4,5-T	TOTALS
Missouri River at Nebraska City, Nebr.	0	2	0	6	6	0	0	0	2	0	0	0	10	1	3	30
Yellowstone River near Billings, Mont.	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	4
James River at Huron, S. Dak.	0	0	0	0	0	0	0	0	3	0	0	0	20	0	3	26
Platte River at Brady, Nebr.	0	0	0	1	0	0	0	0	0	0	0	0	3	1	1	6
Arkansas River below John Martin Reservoir, Colo.	0	3	2	4	5	0	0	0	3	0	0	0	3	1	3	24
Arkansas River at Van Buren, Ark.	0	3	1	9	1	0	0	0	1	0	0	0	5	0	24	44
Canadian River near Whitefield, Okla.	0	1	0	3	0	0	0	0	2	0	0	0	2	1	27	36
Brazos River at Richmond, Tex.	0	6	11	10	2	0	0	0	1	0	0	0	6	0	13	49
Colorado River at Wharton, Tex.	1	4	3	7	1	0	0	0	1	0	0	0	2	0	7	26
Rio Grande below Anzalduas Dam, Tex.	0	2	6	3	0	0	0	0	1	0	0	0	0	1	2	15
Pecos River near Artesia, N. Mex.	0	1	1	4	1	0	0	0	4	1	0	0	3	2	2	19
Colorado River (Yuma Main Canal) at Yuma, Ariz.	0	0	0	1	0	0	0	0	0	0	0	0	2	1	2	6
Green River at Green River, Utah	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	3
Gila River at Gillespie Dam, Ariz.	0	18	33	17	12	3	0	0	21	0	0	1	2	3	1	111
Humboldt River near Rye Patch, Nev.	0	0	0	1	0	0	0	0	0	0	0	0	1	29	1	32
Sacramento River at Verona, Calif.	0	0	0	1	0	0	0	0	0	0	0	0	5	3	5	14
Yakima River at Kiona, Wash.	0	6	7	12	7	0	0	0	0	0	0	0	20	0	4	56
Snake River at King Hill, Idaho	0	1	0	13	1	0	0	0	1	0	0	0	11	0	3	30
Columbia River at The Dalles, Oreg.	0	0	0	1	0	0	0	0	0	0	0	0	3	0	1	5
Feather River at Oroville, Calif.	0	0	0	1	0	0	0	0	0	0	0	0	2	2	5	10
TOTALS	1	47	64	96	36	3	0	0	40	1	0	1	103	45	109	546

NOTE: Only the occurrences of pesticides looked for during the entire study period, October 1968-September 1971, are included in this table.

APPENDIX

*Chemical Names of Compounds Discussed in This Issue**

LDRIN	Not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4- <i>endo-exo</i> -5,8-dimethanonaphthalene
RSENIC	As
HC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
ADMIUM	Cd
HLORDANE	1,2,4,5,6,7,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane
4-D	2,4-dichlorophenoxyacetic acid
DE	1,1-dichloro-2,2-bis(<i>p</i> -chlorophenyl) ethylene
DT (including its isomers and dehydrochlorination products)	1,1,1-trichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane; technical DDT consists of a mixture of the <i>p,p'</i> -isomer and the <i>o,p'</i> -isomer (in a ratio of about 3 or 4 to 1)
IAZINON	<i>O,O</i> -diethyl <i>O</i> -(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate
IELDRIN	Not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8a-octahydro-1,4- <i>endo-exo</i> -5,8-dimethano=naphthalene
NDSULFAN (THIODAN®)	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide
NDRIN	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4- <i>endo-endo</i> -5,8-dimethanonaphthalene
CB	hexachlorobenzene
EPTACHLOR	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
EPTACHLOR EPOXIDE	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane
EAD	Ph
INDANE	1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer
IALATHION	diethyl mercaptosuccinate, <i>S</i> -ester with <i>O,O</i> -dimethyl phosphorodithioate
IERCURY	Hg
IEHYL PARATHION	<i>O,O</i> -dimethyl <i>O-p</i> -nitrophenyl phosphorothioate
IREX	dodecachlorooctahydro-1,3,4-metheno-1 <i>H</i> -cyclobuta[<i>cd</i>]pentalene
ARATHION	<i>o, o</i> -diethyl <i>o-p</i> -nitrophenyl phosphorothioate
OIYCHLORINATED BIPHENYLS (PCB's)	Mixtures of chlorinated biphenyl compounds having various percentages of chlorination
ILVEX	2-(2,4,5-trichlorophenoxy)propionic acid
4,5-T	2,4,5-trichlorophenoxyacetic acid
DE (DDD) (including its isomers and dehydrochlorination products)	1,1-dichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane; technical TDE contains some <i>o,p'</i> -isomer also
OXAPHENE	chlorinated camphene containing 67-69% chlorine

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RESIDUES IN FOOD AND FEED

Residues of Mirex and Certain Other Chlorinated Hydrocarbon Insecticides in Beef Fat—1971¹

J. H. Ford, J. C. Hawthorne, and G. P. Markin

ABSTRACT

Samples of fat from beef cattle were obtained in areas of Mississippi and Georgia where the insecticide mirex had been used to control the imported fire ant. Mirex residues were detected in 67 of the 77 fat samples analyzed with levels ranging from 0.001 (lowest level of detection) to 0.125 ppm, with an average residue level of 0.025 ppm. Sixty-nine samples of fat from beef cattle from nonmirex treated areas were also analyzed; none contained mirex. All fat samples contained residues of DDT.

Introduction

Mirex in a bait form is at present the recommended method of controlling the imported fire ant, *Solenopsis invicta* (Buren) (2), which is a serious agricultural pest in nine southeastern States (1). Since mirex is a very stable chlorinated hydrocarbon insecticide (4) capable of being stored in fat, cattle grazing on treated pastures would possibly accumulate residues of this insecticide. This study was undertaken by the U. S. Department of Agriculture as part of a series of studies to determine the environmental distribution of mirex following application.

Sample Collection

The samples of beef fat for this study were taken from cattle from two areas, heavily infested with fire ants,

which were known from USDA records to have received blanket applications of mirex at least twice in the past 3 years. The areas included the northeastern Mississippi region of Oktibbeha, Prentiss, Lec, Lowndes, and Itawamba Counties, and central Georgia between Macon and Tifton. USDA inspectors collected information on the specific animals from which samples were collected and the treatment history of the pertinent areas.

Since the normal method of residue analysis for meats is to utilize the fat, State meat inspectors were requested to collect 1,000-g samples of subcutaneous fat from five different locations on the freshly-dressed carcass. These five samples were combined into a single composite sample for the animal (1,000 g), wrapped in aluminum foil, and frozen; ½ kg of this composite sample was sent to the Environmental Monitoring Laboratory in Gulfport, Miss., for processing. Since this program was conducted in cooperation with the respective State Departments of Agriculture, a ½ kg of each sample was also sent to the respective State laboratory for replicate analysis.

Samples were also obtained in a similar manner from cattle in check areas outside the region where mirex had been used. The data on individual animals, however, were not as complete. Verification that the animal had come from outside the treated area was obtained from the State meat inspector.

¹ U. S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine Programs, Environmental Quality Laboratory, P. O. Box 989, Gulfport, Miss. 39501.

Analytical Procedures

METHOD OF EXTRACTION

Twenty grams of thoroughly mixed rendered fat was placed in a 1-qt canning jar containing 100 ml of distilled-in-glass isopropanol. This mixture was then blended for 2-3 minutes on a Lourdes blender assembly; 300 ml of distilled-in-glass hexane was then added to the blended mixture with small portions being used to rinse the blender blades. The jar was sealed with a teflon-lined cap and rotated on a concentric rotator for 2 hours. The extract was filtered through glass wool which was prewashed with distilled-in-glass acetone followed by distilled-in-glass hexane. A 300-ml aliquot of the filtered extract (representing 15 g of sample) was placed in a 1-liter separatory funnel and shaken for 1 or 2 minutes with 250 ml of distilled water. The layers were allowed to separate, and the lower layer containing water and isopropanol was discarded. A total of three successive washings with distilled water was utilized, discarding the lower water layer each time.

The remaining hexane layer was then filtered through a filter tube (42 mm O.D. x 160 mm) containing a glass wool plug and anhydrous Na_2SO_4 and the dried hexane placed in a 500-ml 24/40 S-jointed Erlenmeyer flask. The filter tube was washed with several portions of distilled-in-glass hexane and these washings combined with the hexane extract. A three-ball Snyder column was then attached, and the sample concentrated to 25 ml on an explosion-proof hot plate. The concentrate was quantitatively transferred to a 50-ml graduate cylinder and the volume adjusted to 45 ml.

H_2SO_4 CLEANUP

A 15-ml aliquot (representing 5 g of sample) was placed in a 125-ml separatory funnel and 35 ml of distilled-in-glass hexane added; 10 ml of concentrated H_2SO_4 was added to the sample, and the mixture was first swirled gently and then shaken and the pressure released. The layers were allowed to separate, and the lower acid layer was discarded. This acid wash was repeated, the acid layer again discarded, and the sample then washed three successive times with distilled water, discarding the lower water layer each time. The hexane sample was next filtered again through a filter tube containing anhydrous Na_2SO_4 and prewashed glass wool into a 250-ml 24/40 S-jointed Erlenmeyer flask. The filter tube was washed with several small portions of distilled-in-glass hexane and the washings combined with the sample. A Snyder column was again employed, and the sample concentrated to about 10 ml on the hot plate.

PREPARATION OF THE FLORISIL COLUMN

The chromatographic column employed consisted of a 125-ml reservoir attached to an 11- x 150-mm glass tube,

a removable teflon stopcock, and a removable glass tip. A small piece of hexane-washed glass wool was placed in the bottom of the column and 1 inch of anhydrous Na_2SO_4 added; 18 g of 60/120 mesh PR grade unactivated Florisil was placed in the column with gentle tapping to insure even packing, and another 1-inch layer of Na_2SO_4 was placed atop the Florisil. The column was then prewashed with 100 ml of distilled-in-glass methylene chloride and allowed to drain to the top of the upper layer of Na_2SO_4 . The washing was then repeated with 100 ml of distilled-in-glass hexane. The column was never allowed to go dry. The sample extract was then quantitatively transferred to the Florisil column. When the extract drained to the upper layer of Na_2SO_4 , 100 ml of a 5% by volume mixture of methylene chloride in hexane was added to the column and the sample collected until the eluant level reached the upper Na_2SO_4 level. This elution was labeled fraction one. A second fraction was collected using 100 ml of distilled-in-glass methylene chloride as the eluant. The second fraction was collected until the column ran dry.

Mirex, heptachlor, *p,p'*-DDT and its metabolites, and sometimes heptachlor epoxide appeared in the first fraction; the second fraction was collected because it also sometimes contains heptachlor epoxide. To the collection flasks were added 1 ml of a 0.01% Nujol solution in hexane and three or four glass beads. The Snyder column and hot plate were again employed, and the sample concentrated to about 5 ml. To the second fraction, 50 ml of hexane was added and again concentrated to about 5 ml in order to remove all traces of methylene chloride. Five milliliters of distilled-in-glass hexane was poured through the top of the Snyder column and collected in the flask. This was done to rinse any volatilized pesticide from the column. The sample was then quantitatively transferred to a 15-ml graduated centrifuge tube and placed into a water bath maintained at 40° C. A stream of air was directed into the centrifuge tube until the volume was reduced to 2.5 ml.

GAS CHROMATOGRAPHIC ANALYSIS

Ten microliters of this sample (representing 20 mg of the original fat sample) were normally injected on the instrument. Two different column types, 6 feet in length and 1/4 inch O.D., were utilized as a confirmatory procedure: a mixed column of 1.5% OV-17 and 1.95% QF-1 on 100/120 mesh Gas Chrom Q and a 3% DC-200 column on 100/120 mesh Gas Chrom Q. The mixed column was used in a MicroTek Model MT220; the oven temperature was 200°C, the injection port temperature 250°C, and the detector temperature 210°C. The flow rate was 80 ml/min of a nitrogen carrier gas. The DC-200 column was used in a Hewlett-Packard Model 402; the oven temperature was 175°C,

the injection port temperature 245°C, and the detector temperature 205°C. The flow rate was 100 ml/min of 5% methane-95% argon carrier gas.

Average recovery values of 90% for the fat sample were determined by fortification with composite pesticide standards. All data were corrected for percent recovery.

The presence of polychlorinated biphenyls (PCB's) (Aroclor 1260®, in particular) in some of the samples interfered with the mirex analysis on both GC columns. In these samples the PCB's were separated using a modification (6) of the Armour-Burke method (7).

Results and Discussion

Mirex and certain other chlorinated pesticide residues detected in beef fat from the treated areas are reported in Table 1. Also presented in this table are residues of other tissues (fatty tissue, kidney, liver, and lean portion) from a few specimens. These analyses from our laboratory at Gulfport generally agreed within 10% with those from the State laboratories of Mississippi and Georgia. Residues of mirex were found in 88% of the samples from this area with levels ranging from 0.001 ppm (our lowest level of detection) to 0.125 ppm, with an average value of 0.026 ppm. Only one sample, 0.125 ppm, was in excess of the established tolerance level of 0.1 ppm for mirex in the fat of the beef cattle (4). Analysis of the other tissues showed no residues in the lean portions and very small residues in the fatty tissues from the same animals.

Because mirex has been used previously as a fire ant retardant under the name Dechloran® (5), it was decided to examine samples collected from untreated areas, both

in the Southeast and from other areas. These results are reported in Table 2. The absence of mirex in samples from untreated areas, while not giving absolute proof that the residues found in samples from the treated areas were from the insecticide application, does strongly indicate that this is the case. It does show that prior uses of this compound have not made it ubiquitous in the Nation's environment.

In general, we have concluded that residues can be expected in beef fat of animals in mirex-treated areas following the use of mirex, but that application in the prescribed manner will keep these residue levels below the tolerance level.

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TABLE 1.—Residues of mirex and certain other chlorinated hydrocarbon insecticides in fat from beef cattle in mirex-treated areas—1970

SAMPLE NUMBER	SAMPLE LOCATION	TYPE OF CATTLE	RESIDUES IN PPM		
			MIREX	TOTAL DDT	OTHERS
MISSISSIPPI					
00158	Columbus	Black Angus heifer	0.030	0.41	¹ 0.52
00159	Fremont	Spotted Jersey calf	0.005	0.165	—
00160	Artesia	Angus-Holstein cross	0.016	0.020	¹ 0.64
00161	Artesia	Angus-Holstein cross	0.035	5.0	—
00162	Artesia	Hereford heifer	0.03	7.99	—
00163	Columbus	Angus-Jersey cross	0.011	0.88	—
00164	Columbus	Angus-Hereford cross	0.07	0.27	—
00165	Columbus	Angus-Jersey cross	0.025	1.35	¹ 0.48
00166	Columbus	Whiteface heifer	0.01	0.271	—
00167	Columbus	Whiteface steer	0.038	0.243	—
00168	Columbus	Whiteface heifer	0.026	0.24	—
00169	Columbus	Angus-Jersey cross	0.04	1.19	² 0.51
00170	Columbus	Whiteface steer	0.02	0.159	—
00218	Starkville	Jersey bull calf			
		fatty tissue	0.029	0.245	—
		kidney	—	0.007	—
		liver	—	0.005	—
00219	Starkville	Guernsey bull calf			
		fatty tissue	0.024	0.314	—
		kidney	0.001	0.011	—
		liver	—	0.006	—
		lean portion	—	0.011	—
00220	Starkville	Jersey bull calf			
		fatty tissue	0.029	0.206	—
		kidney	0.002	0.005	—
		liver	—	0.007	—
		lean muscle	—	0.004	—
00292	Columbus	Hereford-Jersey cross	0.005	0.838	—
00293	Baldwin	Hereford	0.036	0.65	—
00294	Baldwin	Angus	0.008	0.786	—
00295	Baldwin	Angus	0.02	0.519	—
00296	Saltillo	Angus bull	0.02	0.370	—
00297	Saltillo	Angus steer	0.05	0.72	—
GEORGIA					
00267	Alma (Bacon Co.)	Cow	0.018	0.429	—
00268	Alma (Bacon Co.)	Steer	0.005	0.121	—
00269	Nicholls (Coffee Co.)	Steer	0.017	0.109	—
00270	Waycross (Ware Co.)	Steer	0.003	0.046	—
00271	Dublin (Laurens Co.)	Bull	0.002	0.131	—
00272	Dudley (Laurens Co.)	Bull	0.041	3.906	—
00273	Dublin (Laurens Co.)	Heifer	0.010	0.634	—

TABLE 1.—*Residues of mirex and certain other chlorinated hydrocarbon insecticides in fat from beef cattle in mirex-treated areas—1970—Continued*

SAMPLE NUMBER	SAMPLE LOCATION	TYPE OF CATTLE	RESIDUES IN PPM		
			MIREX	TOTAL DDT	OTHERS
GEORGIA—Continued					
00274	Dublin (Laurens Co.)	Heifer	0.050	0.547	—
00275	Glenwood (Laurens Co.)	Steer	0.041	2.081	—
00276	Forsyth (Monroe Co.)	Dairy cow	0.013	0.102	—
00277	Forsyth (Monroe Co.)	Dairy cow	0.002	0.063	—
00298	Patterson (Pierce Co.)	Steer	0.020	0.127	—
00299	Screven (Wayne Co.)	Heifer	0.001	0.160	^a 0.11
00300	Waycross (Ware Co.)	Steer	0.061	0.308	^a 0.07
00301	Waycross (Ware Co.)	Heifer	0.073	0.122	—
00302	Hoboken (Brantley Co.)	Heifer	0.096	0.156	—
00303	Guyton (Effingham Co.)	Steer	0.028	0.091	—
00304	Guyton (Effingham Co.)	Steer	0.040	0.120	—
00305	Guyton (Effingham Co.)	Steer	0.033	0.11	—
00306	Guyton (Effingham Co.)	Steer	0.060	0.075	—
00307	Savannah (Chatham Co.)	Steer	0.013	0.081	—
00308	Hortense (Brantley Co.)	Steer	0.002	1.685	—
00320	Statesboro (Bulloch Co.)	Heifer	0.047	0.329	—
00321	Statesboro (Bulloch Co.)	Heifer	—	0.169	—
00322	Statesboro (Bulloch Co.)	Steer	0.010	0.138	—
00323	Statesboro (Bulloch Co.)	Steer	0.015	0.367	—
00324	Millwood (Ware Co.)	Steer	0.052	0.136	—
00325	Alma (Bacon Co.)	Steer	0.125	0.469	—
00326	Cordele (Crisp Co.)	Steer	—	0.613	—
00327	Cordele (Crisp Co.)	Steer	—	0.446	^a 0.48
00328	Vienna (Crisp Co.)	Steer	—	0.910	—
00329	Rochelle (Wilcox Co.)	Steer	0.017	1.921	^a 0.52
00330	Statesboro (Bulloch Co.)	Cow	0.053	0.202	—
00331	Statesboro (Bulloch Co.)	Steer	0.020	0.457	—
00472	Statesboro (Bulloch Co.)	Steer	0.087	1.611	—
00473	Statesboro (Bulloch Co.)	Steer	0.091	0.051	—
00474	Statesboro (Bulloch Co.)	Steer	—	0.037	—
00475	Statesboro (Bulloch Co.)	Steer	—	0.135	—
00486	Guyton (Effingham Co.)	Steer	0.029	0.059	—
00487	Brooklet (Bulloch Co.)	Steer	0.004	0.328	—
00488	Cordele (Crisp Co.)	Steer	0.024	0.130	—
00489	Pincora (Chatham Co.)	Steer	—	0.081	—
00490	Savannah (Chatham Co.)	Steer	0.016	0.281	—
00491	Pincora (Chatham Co.)	Steer	0.003	0.256	—
00492	Statesboro (Bulloch Co.)	Steer	0.002	0.074	—
00706	Atlanta (Fulton & DeKalb Co.)	Steer	0.041	0.129	—
00707	Guyton (Effingham Co.)	Heifer	0.040	0.286	—
00708	Savannah (Chatham Co.)	Steer	—	0.254	—
00709	Pincora (Chatham Co.)	Steer	0.022	0.576	—

TABLE 1.—Residues of mirex and certain other chlorinated hydrocarbon insecticides in fat from beef cattle in mirex-treated areas—1970—Continued

SAMPLE NUMBER	SAMPLE LOCATION	TYPE OF CATTLE	RESIDUES IN PPM		
			MIREX	TOTAL DDT	OTHERS
GEORGIA—Continued					
00710	Statesboro (Bulloch Co.)	Steer	—	0.209	—
00711	Forsyth (Monroe Co.)	Cow	0.020	1.472	—
00712	Guyton (Effingham Co.)	Steer	0.023	0.171	—
00713	Gray (Jones Co.)	Cow	0.032	0.118	—
00714	Haddock (Jones Co.)	Cow	0.019	0.323	—
00715	Snarr (Monroe Co.)	Cow	—	0.054	—

NOTE: — = not detected.

¹ Aroclor 1254®.

² Aroclor 1260®.

³ Technical chlordane.

TABLE 2.—Residues of mirex and certain other chlorinated hydrocarbon insecticides in fat from beef cattle in areas outside the mirex-treated region—1970

SAMPLE NUMBER	SAMPLE LOCATION	TYPE OF CATTLE	RESIDUES IN PPM		
			MIREX	TOTAL DDT	OTHERS
00476	Iowa	(1)	—	0.02	—
00477	Iowa		—	—	—
00478	Iowa		—	0.06	—
00479	Iowa		—	—	—
00480	Iowa		—	—	—
00485	Iowa		—	0.019	—
00481	Nebraska		—	0.01	—
00482	Nebraska		—	0.012	—
00483	Illinois		—	0.119	—
00484	Nebraska		—	0.022	—
	Mississippi				
00486	Batesville		—	0.24	—
00847	Marks		—	0.43	—
00848	Batesville		—	0.35	—
00849	Crowder		—	0.32	—
00850	Pope		—	0.18	—
00851	Como		—	0.19	—
00852	Marks		—	0.29	—
00853	Crowder		—	0.042	—
00854	Belen		—	0.26	—
00855	Charleston		—	0.50	—

TABLE 2.—*Residues of mirex and certain other chlorinated hydrocarbon insecticides in fat from beef cattle in areas outside the mirex-treated region—1970—Continued*

SAMPLE NUMBER	SAMPLE LOCATION	TYPE OF CATTLE	RESIDUES IN PPM		
			MIREX	TOTAL DDT	OTHERS
	South Carolina				
00880	Darlington		—	0.590	—
00881	Darlington		—	0.703	—
00882	Darlington		—	0.160	—
00883	Dillon		—	0.530	—
00884	Dillon		—	1.440	—
00885	Darlington		—	1.320	—
00886	Dillon		—	5.800	—
	Arkansas				
00980	Pollard	Heifer	—	0.145	—
00981	Piggott	Bull	—	0.105	—
00983	Pollard	Heifer	—	0.620	—
00984	Pollard	Heifer	—	0.085	—
00985	Greene Co.	Heifer	—	0.220	—
00986	Paragould	Bull	—	0.038	—
00987	Greene Co.	Bull	—	0.190	—
00988	Paragould	Bull	—	0.190	—
00982	Fremont, Mo.	Bull	—	0.074	—
	Tennessee				
00856	Union City	Heifer	—	0.067	—
00857	Dyersburg	Heifer	—	0.03	—
00858	Union City	Bull	—	0.058	^a 0.11
00859	Union City	Heifer	—	0.051	—
00860	Union City	Heifer	—	0.06	—
00861	Dyersburg	Bull	—	0.054	—
00862	Dyersburg	Heifer	—	0.039	—
00863	Dyersburg	Bull	—	0.05	—
01024	Lynnville	Heifer	—	0.04	—
01025	Lynnville	Bull	—	0.043	—
01026	Lynnville	Heifer	—	0.21	—
01027	Lynnville	Heifer	—	0.16	—
01028	Lynnville	Heifer	—	0.20	—
	Georgia				
01029	Washington Co.	Bull	—	0.95	—
01030	Social Circle	Heifer	—	0.06	—
01031	Social Circle	Bull	—	0.31	—
01032	Monroe	Bull	—	0.11	—
01033	Green Co.	Bull	—	0.35	—
01034	Rockdale Co.	Heifer	0.01	0.08	—
01035	Walton Co.	Bull	—	0.21	—
01036	Eatonton	Bull	—	0.074	—
01037	Eatonton	Bull	—	0.056	—
01038	Eatonton	Bull	—	0.106	—

TABLE 2.—*Residues of mirex and certain other chlorinated hydrocarbon insecticides in fat from beef cattle in areas outside the mirex-treated region—1970—Continued*

SAMPLE NUMBER	SAMPLE LOCATION	TYPE OF CATTLE	RESIDUES IN PPM		
			MIREX	TOTAL DDT	OTHERS
	North Carolina				
01039	Mt. Olive	Dairy cow	—	0.78	—
01040	Mt. Olive	Dairy cow	—	0.42	—
01041	Beaulaville	Bull	—	0.55	—
01042	Beaulaville	Bull	—	0.30	—
01043	Kenansville	Bull	—	0.29	—
01044	Burgaw	Bull	—	0.07	—
01045	Burgaw	Bull	—	0.04	—
01046	Willand	Heifer	—	0.11	—
01047	Wallace	Bull	—	0.98	—
01048	Wallace	Bull	—	0.31	—

NOTE: — = not detected.

¹ Blank = type of cattle unknown.

² Aroclor 1254®.

Residues of PCB's and PCT's in Canadian and Imported European Cheeses, Canada—1972

David C. Villeneuve,¹ Lincoln M. Reynolds,² and William E. J. Phillips¹

ABSTRACT

Twenty Canadian and twenty imported European cheese samples were analyzed for PCB and PCT residues. The average PCB residue, determined on a whole-weight basis, in Canadian cheese was 0.042 ppm (range 0.01-0.27 ppm), while the average residue in imported cheese was 0.043 ppm (range 0.02-0.08 ppm). None of the cheese samples contained PCT residues at or above the detectable limit of the method (0.005 ppm).

Introduction

During 1972 an extensive survey was carried out to determine the levels of polychlorinated biphenyls (PCB's) and polychlorinated terphenyls (PCT's) in food packaging materials (1). Although cheese products were included in this survey, only a limited number of samples were obtained, and all were of the dry variety. Since none of these samples were packaged in material that contained ≥ 10 ppm of PCB's or PCT's, no cheese sample was actually analyzed for PCB's or PCT's. The present survey, carried out in 1972, was designed to determine the PCB and PCT content of cheeses, both domestic and imported, that are available to the Canadian consumer.

Sampling Procedures

Samples of cheese representing 31 different companies were obtained by Health Protection Branch Inspectors in five centers across Canada: the Eastern region, representing the Maritime Provinces, and the Quebec, Ontario, Manitoba, and Vancouver regions. Four domestic cheeses and four imported cheeses were selected from each region and were forwarded to the Ontario Research Foundation for analysis. The types of cheeses included the following: Cheddar, Mozzarella, Caciocavallo, Ricotta, Blue, Gouda, Fynbo, Tilsit, Havarti, Kasserl, Camembert, Semisoft, Lancashire, Butter, Esrom, Ambrosia, and Noekkelost.

Analytical Procedures

Analyses of the cheese samples for PCB's and PCT's were carried out as follows: The cheese sample (1-2 lb) was mascerated, and a 10-g representative aliquot, two tablespoons of anhydrous sodium sulfate, and 75 ml of 50% benzene in acetone were blended in a Sorval® mixer for 1 minute. The extract was filtered through a jacketed funnel with hot water being circulated to keep the fat in solution. The blender jar and filtering funnel were rinsed with benzene/acetone until 150 ml was collected. This solution was chilled in a dry ice/methanol bath for 3 minutes and the flocculent precipitate obtained was filtered off through sodium sulfate rinsing with 50 ml of the benzene/acetone solution. The filtrate was then concentrated and put through a Florisil

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cleanup and then subjected to a "PCB split" (2). The GLC parameters for the determination of PCB and PCT residues are described elsewhere (1). Recoveries for PCB's and PCT's were 90% and 85%, respectively; results were not corrected for percent recovery. The limit of detection for both PCB's and PCT's was 0.005 ppm. Because low levels of PCB's were found, confirmation was not carried out.

Results and Discussion

The results obtained for Canadian cheese samples are shown in Table 1. Only one sample contained PCB's above 0.1 ppm, and the average content was 0.042 ppm (whole-weight basis). No cheese sample contained PCT's at or above the detectable limit of the method (0.005

ppm). The results obtained for imported cheese samples are shown in Table 2. No imported cheese sample contained PCB's above 0.1 ppm (average = 0.043 ppm) and as with the Canadian cheese samples, none contained any detectable amount of PCT's.

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TABLE 1.—Results of analyses for PCB's and PCT's in Canadian cheese samples

SAMPLE SOURCE	RESIDUES IN PPM, WHOLE-WEIGHT BASIS	
	PCB's	PCT's
Montreal, Quebec	0.06	—
	0.27	—
	0.04	—
	0.05	—
Winnipeg, Manitoba	0.01	—
	0.03	—
	0.03	—
	0.02	—
Vancouver, British Columbia	0.02	—
	0.02	—
	0.04	—
	0.01	—
Charlottetown, Prince Edward Island	0.02	—
	0.01	—
Saint John, New Brunswick	0.04	—
Halifax, Nova Scotia	0.05	—
Toronto, Ontario	0.03	—
	0.04	—
	0.01	—
	0.03	—

NOTE: — = Not detected at or above the limit of the method (0.005 ppm).

TABLE 2.—Results of analyses for PCB's and PCT's in imported European cheese samples

COUNTRY OF ORIGIN	RESIDUES IN PPM, WHOLE-WEIGHT BASIS	
	PCB's	PCT's
Denmark	0.03	—
	0.04	—
	0.05	—
	0.03	—
	0.03	—
Holland	0.03	—
	0.02	—
	0.05	—
Greece	0.04	—
	0.08	—
	0.02	—
France	0.06	—
England	0.03	—
	0.02	—
West Germany	0.05	—
	0.07	—
Sweden	0.04	—
Norway	0.05	—
	0.05	—
Switzerland	0.06	—

NOTE: — = Not detected at or above the limit of the method (0.005 ppm).

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Organochlorine Residues in Wild Moose, Idaho—1972¹

W. W. Benson, Michael Watson, and Joe Wyllie

ABSTRACT

*In 1972, samples of adipose tissue from 14 moose, *Alces alces shirasi*, from the Targhee National Forest in eastern Idaho were analyzed by gas-liquid chromatography for organochlorine residues. Residues detected and respective mean levels for each were: p,p'-DDT, 52.3 ppb; p,p'-DDE, 28.9 ppb; p,p'-DDD, 53.0 ppb; α -BHC, 77.5 ppb; and dieldrin, 5.0 ppb. The presence of significant BHC levels in these animals probably reflects the prior use of BHC within the forest for control of pine bark beetle infestation.*

Introduction

Pesticide residues and other contaminants continue to be found in many forms of wildlife. Although tissues from most of the wild North American ungulates of the deer family have been examined in detail for pesticide residue levels (1-6), there is little information regarding the extent of these contaminants in moose. To our knowledge, the only other report of pesticides in these animals is that of Walker *et al.* in 1965 (2) in which adipose tissues from three bull moose taken in central Idaho in 1962 were analyzed for DDT residues.

This study reports organochlorine residues in the adipose tissue of 14 wild moose, *Alces alces shirasi*, taken during the 1972 hunting season in the Targhee National Forest in eastern Idaho. Several areas of the forest are infested with the mountain pine bark beetle, *Dendroctonus ponderosii*, and were sprayed from 1963 through

1970 to control the infestation. This study was undertaken, as part of our wildlife monitoring program, in order to determine current body burdens of organochlorines in moose that may have resulted following the various spraying episodes.

Sampling and Analytical Procedures

Moose in Idaho are a protected species, occurring only in limited areas. They are hunted only on a strictly controlled basis by means of special permits issued each fall by the Idaho Department of Fish and Game; in 1972, 2,923 hunters applied for the 120 available permits (1973, J. Graben, Idaho Dept. of Fish and Game, *personal communication*). Thus, sampling techniques were dependent on and limited to these circumstances, and a more statistically desirable sampling procedure was not possible.

Although moose flesh is esteemed as table fare, they are primarily a trophy species; consequently, the large, antlered males are usually the animal of choice. To facilitate proper game management, all successful hunters in Idaho are required to have their big game checked by the local conservation officer. Through the cooperation of the Idaho Fish and Game Department, samples of subcutaneous adipose tissue were obtained from 12 male and 2 female adult moose at checking stations in hunting areas 60, 61, and 62 as established by the Department of Fish and Game. The kill locations were all within a 25-mile radius of one another and were almost entirely within the boundaries of the Targhee National Forest in Fremont County, Idaho. Tissues were collected during October 1972, transported immediately to the Idaho Community Study on Pesticides Laboratory in Boise, and frozen at -4°C until analyzed in December 1972.

¹ From the Idaho Community Study on Pesticides, Idaho Department of Environmental and Community Services, Statehouse, Boise, Idaho 83702.

Five-gram portions of each adipose tissue sample were extracted with petroleum ether and partitioned with acetonitrile according to the basic method of de Faubert Maunder *et al.* (7). Column cleanup on Florisil followed the procedure of Mills *et al.* (8, 9). Analysis was by means of a MicroTek 220 gas chromatograph, equipped with two differing columns and tritium foil electron capture detectors. This dual column technique was used in order to provide a reliable means of confirmatory analysis. In the case of α -BHC residues, all determinations were subjected to additional gas-chromatographic confirmation following TLC migration.

Columns:	4% SE-30/6% QF-1 on 80/100 mesh Chromosorb W, DCMS	
	1.5% OV-17/1.95% QF-1 on 100/120 mesh Chromosorb W, DCMS	
Temperatures:	Columns	200° C
	Injection chamber	220° C
	Detector	205° C
Carrier gas:	Nitrogen	
Carrier gas flow:	On SE-30/QF-1 column	90 ml/min
	On OV-17/QF-1 column	70 ml/min

This procedure, used extensively in our laboratory for several years, is easily capable of detecting pesticide residues in a 5-g sample at the parts per trillion (ppt) level. For convenience, however, any pesticide levels obviously less than one part per billion (ppb) were considered negative. Percent recovery ranged from 82% to 100% and was based on the addition, prior to extraction, of a known amount of aldrin (Octalene®) to each sample as an internal standard.

Results and Discussion

The pesticide residues detected in the moose adipose samples are shown in Table 1; no polychlorinated biphenyls (PCB's) were noted in any of the samples tested. It is worthy of note that all residue levels detected are well below the minimum EPA tolerance levels for beef fat. *P,p'*-DDT and its metabolite *p,p'*-DDE were found in all 14 animals tested. *P,p'*-DDD was detected in only two samples. All but one of the moose had higher levels of *p,p'*-DDT than *p,p'*-DDE and the mean level for DDT (52.3 ± 7.0 ppb) was significantly higher than the mean DDE level (28.9 ± 5.7 ppb). This is consistent with previous findings in other wild members of the deer family (1-6). However, it is the reverse of the situation normally found in humans, in which DDE residues are usually considerably higher than are DDT levels (10). In our area, this trend has been well documented by recent large-scale monitoring of human tissues in both Idaho (11, 12) and Utah (13). It is possible that the different mode of digestion and intestinal flora of these wild ruminants are responsible for this interesting variance.

TABLE 1.—Summary of organochlorine residues detected in moose adipose tissue, Idaho—1972

SAMPLED NUMBER	SEX	RESIDUES IN PPB				
		<i>p,p'</i> -DDT	<i>p,p'</i> -DDE	<i>p,p'</i> -DDD	α -BHC	DIELORIN
1	M	19	20	8	—	2
2	M	131	98	98	—	24
3	M	44	26	—	115	5
4	M	33	19	—	33	5
5	M	46	19	—	57	5
6	M	47	20	—	56	—
7	M	47	26	—	48	2
8	M	56	32	—	64	1
9	M	73	35	—	130	2
10	M	38	16	—	124	—
11	M	53	22	—	124	3
12	M	62	41	—	71	—
13	F	41	13	—	47	2
14	F	42	17	—	61	4
Observed Range		112	85	90	97	22
Median		47	22	—	61	3
Mean $\pm$ S.E.		52.3 ± 7.0	28.9 ± 5.7	53.0 ± 17.0	77.5 ± 9.4	5.0 ± 1.7

NOTE: — = no residue detected; no polychlorinated biphenyl residues were detected in any samples.

The widespread occurrence of α -BHC in the moose is also somewhat surprising. From 1963 through 1966 and subsequently from 1969, ethylene dibromide has been the chief insecticide used in the Targhee National Forest (1973, D. T. Fluckiger, Branch Chief, Targhee National Forest, *personal communication*). During 1967 and 1968, γ -BHC (lindane), β -BHC, and cacodylic acid were applied on a limited basis, but the use of BHC was discontinued in 1969 because of objectionable features. The sites treated with BHC during the 2-year period consisted of 31 campground areas comprising a total of 369 acres; a total of 96 lb. was applied. Although moose have been occasionally observed near the treated campground sites, such public areas are usually not frequented by the herd (1973, D. T. Fluckiger, Branch Chief, Targhee National Forest, *personal communication*). Moreover, the vegetation types within the areas treated with BHC do not provide an abundance of forage for moose at any time of the year. However, since a large portion of the diet of these animals in their summer range consists of underwater vegetation, it is possible that additional pesticides were transmitted to these forage areas by adjacent runoff. In spite of these factors, the prior use of BHC for control of pine bark beetles is the only plausible explanation for its occurrence. Perhaps even more perplexing is the occurrence of

the alpha isomer of BHC in the moose, but not the β - or the γ - (lindane) forms. Since only β - and γ -BHC were sprayed, the occurrence of the alpha form in the animals is quite intriguing from a metabolic standpoint.

Since 1965 the use of aldrin and dieldrin in Idaho has been restricted to certain specific purposes. In spite of this, dieldrin was detected in 11 of the 14 animals (Table 1). However, only one moose had adipose dieldrin concentrations that were appreciably high, suggesting that dieldrin is probably not a major body burden component within the herd. In 1965, the report of Walker *et al.* (2) found mean adipose DDT levels of 87 ppb and mean DDE levels of 10 ppb in three moose taken in 1962 from north central Idaho. These levels were slightly higher than our mean for DDT and slightly lower than our mean for DDE (Table 1), but higher DDT levels might be expected during those years of more extensive environmental DDT dispersal.

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See Appendix for chemical names of compounds discussed in this paper.

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Organochlorine Residues in Woodcock Wings, 11 States—1970-71

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Donald L. Hughes³

ABSTRACT

A survey of organochlorine residues in woodcock wings was undertaken to determine whether these wings are suitable for showing regional differences in residues and to obtain a baseline in 1970-71 for later comparisons. Woodcock wings were obtained from the annual hunter's wing survey. Samples came from eight States (Louisiana, Maine, Michigan, New Hampshire, New Jersey, New York, Pennsylvania, and Wisconsin) and one tri-State area (North Carolina, South Carolina, and Georgia). Wings from the tri-State area contained significantly higher ($P < 0.01$) concentrations of DDT (including DDT, DDD, and DDE) than those from other States. Concentrations of polychlorinated biphenyls (PCB's) also were significantly higher ($P < 0.05$) in samples from these three States. Wings from Louisiana and the tri-State area had significantly higher ($P < 0.01$) concentrations of dieldrin than wings from the other States, and those from Louisiana had significantly higher ($P < 0.01$) concentrations of mirex than those from other States.

The compounds detected and the ranges of residue means for all sampling areas were as follows: Total DDT (5.89 — 65.15 ppm); DDT (0.34 — 14.93 ppm); DDE (4.66 — 47.47 ppm); DDD (0.11 — 3.44 ppm); mirex (0.76 — 16.93 ppm); dieldrin (0.09 — 3.06 ppm); and PCB's (4.27 — 8.63 ppm).

Woodcock wings appear to be suitable for determining regional differences in organochlorine residues in this species.

Introduction

More than 1 million woodcock (*Philohela minor*) were harvested in the eastern United States during the 1970-71 fall migrations (2). Because these birds subsist on animal material, primarily earthworms (5, 8), they can be used to measure pollution in an important terrestrial food chain. Their propensity to accumulate residues from their foods was clearly shown during the 1950's when heptachlor was used throughout the Southeast in an effort to control the imported fire ant (*Solenopsis saevissima*). Application of heptachlor to the land resulted in the widespread occurrence of heptachlor epoxide in the tissues of many species of birds, including woodcock (3, 7). Woodcock wintering in the South accumulated residues from their food and carried them north with them in the spring (9). Residues declined following discontinuation of the program (6).

The successful use of waterfowl wings to measure pesticide levels in black ducks (*Anas rubripes*) and mallards (*Anas platyrhynchos*) (4) suggested the possibility that woodcock wings might be similarly employed.

Woodcock wings are obtained from hunters annually to evaluate the status and reproductive success of this species (2). The usefulness of wings in pesticide measurements was explored in 1970-71 by taking subsamples of these wings. This paper reports the results of this sampling.

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Methods

Wing samples were taken from 11 States: Georgia, Louisiana, Maine, Michigan, New Hampshire, New Jersey, New York, North Carolina, Pennsylvania, South Carolina, and Wisconsin. These States were selected to include those that had the largest woodcock harvest and to include states from both North and South. Wings from North Carolina, South Carolina, and Georgia were combined into one sample because there were too few wings to provide a sample from each State.

Wings from each State and from the tri-State area were systematically sorted into groups of 25 for each State. Five of these groups were randomly selected for analysis from each State except for Louisiana and the tri-State area from which there were enough wings for only 4 groups.

Wings were prepared for analysis by plucking the feathers and removing the distal joint. The remaining portion was ground in a hand grinder and homogenized in the groups of 25 that constituted the sample. A 20-g aliquot was taken for analysis.

Analyses for organochlorine pesticides and polychlorinated biphenyls (PCB's) were made at WARF Institute, Inc., Madison, Wis., by the following procedures:

The 20-g aliquot was dried at 40°C for 72-96 hours, then ground with sodium sulfate and extracted with a mixture of ethyl and petroleum ether (30:70) for 8 hours in a Soxhlet apparatus. The extract was cleaned and separated into two fractions by elution through a Florisil column with mixtures of ethyl and petroleum ether (5:95 and 15:85). An aliquot of the first elution was passed through a standardized silicic acid column with petroleum ether, hexane, acetonitrile, and methylene chloride as described by Armour and Burke (1).

Analysis was by electron capture gas chromatography on a Barber-Coleman Pesticide Analyzer Model 5360. Instrument conditions were:

- (1) Column, 4 ft. x 4 mm glass, packed with 5% DC-200 on 80/100 mesh Gas Chrom Q; injector temperature 240°C, column 200°C, and detector 230°C; flow such that *p,p'*-DDT had a retention time of 6-8 minute.
- (2) Column, 4 ft. x 4 mm glass, packed with 3% OV-17 and 100/120 mesh Gas Chrom Q; injector temperature 230°C, column 185°C, and detector 250°C; flow such that lindane had a retention time of 1 minute.

This method detected organochlorines at a sensitivity level of 0.05 ppm. No corrections were made for recoveries, which were 85% or better.

Lipids were determined on an aliquot of the extract reduced to dryness on a steam bath and placed in a 40°C oven for 2-4 hours. All residues are expressed on a lipid-weight basis because the relative amounts of bone, feather, and moisture in the samples were uncontrolled variables. The presence of mirex was confirmed by mass spectrometry by W. L. Reichel at Patuxent Wildlife Research Center, Laurel, Md.

Results and Discussion

Table 1 shows the ranges and means for DDT, DDE, DDD, mirex, dieldrin, and PCB's for the eight States and the tri-State area. DDT and its metabolites exhibited the same overall pattern: The mean residues of DDT and each metabolite were significantly higher ($P < 0.01$) in the tri-State area than in other States (DDT, 14.93 ppm; DDE, 47.47 ppm; and DDD, 3.44 ppm) and New Jersey woodcock wings contained the next highest concentrations of these residues; DDT concentrations were significantly higher ($P < 0.01$) than in other States, but DDE and DDD residues were not significantly higher ($P < 0.05$). Figure 1 shows the overall pattern of the total DDT residues (including DDT, DDE, and DDD) in the States from which wings were analyzed.

The mean concentrations of dieldrin were significantly higher ($P < 0.01$) in woodcock wings from Louisiana (2.37 ppm) and from the tri-State area (3.06 ppm) than in those from any of the northern States (Fig. 2). Wings from Louisiana and the tri-State area also had higher concentrations of mirex (16.93 ppm and 4.08 ppm, respectively) although only those from Louisiana were significantly higher ($P < 0.01$) than the others (Fig. 3).

PCB's were found in all samples; the means ranged from 4.27 ppm in Maine to 8.63 ppm in the tri-State area. The PCB concentrations were significantly higher ($P < 0.05$) in the tri-State area than all other States.

The results of this sampling of wings of all populations of woodcock for organochlorine residues (DDT, DDE, DDD, mirex, dieldrin, and PCB's) indicate clear geographic differences in residue levels. Woodcock wings appear useful for determining regional differences in organochlorine residues and for following their trends.

See Appendix for chemical names of compounds discussed in this paper.

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TABLE 1.—Organochlorines in woodcock wings from 11 states—1970-71

STATE	RESIDUES IN PPM, LIPID-WEIGHT BASIS						
	TOTAL DDT ¹	DDT	DDE	DDD	MIREX	DIELDRIN	PCB'S
Pennsylvania							
range	2.57-10.15	0.27-1.83	2.21-8.01	0.09-0.31	0.71-2.23	0.08-0.11	3.82-5.31
mean	6.49	1.06	5.23	0.19	1.14	0.10	4.67
New Hampshire							
range	3.45-25.18	0.55-3.61	2.84-21.20	0.06-0.36	0.42-1.46	0.10-0.19	4.45-7.39
mean	11.72	2.02	9.50	0.20	0.87	0.13	5.74
New York							
range	5.01-8.07	0.45-0.82	4.50-7.08	0.06-0.17	1.18-2.58	0.13-0.29	4.62-8.61
mean	6.41	0.65	5.64	0.12	1.77	0.20	6.87
Wisconsin							
range	3.60-21.96	0.27-0.52	3.28-21.21	0.05-0.23	0.62-2.40	0.06-0.30	3.79-14.34
mean	10.79	0.34	10.34	0.11	1.41	0.16	7.10
Maine							
range	3.59-10.19	0.48-1.48	3.03-8.40	0.08-0.31	0.57-2.24	0.06-0.15	3.48-4.78
mean	5.89	1.02	4.66	0.21	1.34	0.09	4.27
New Jersey							
range	12.43-46.06	1.91-18.22	10.14-26.70	0.38-1.14	<0.05-1.38	0.26-2.33	2.71-8.09
mean	25.15	8.28	16.20	0.67	0.76	0.95	6.41
Michigan							
range	2.45-17.13	0.46-3.31	1.89-13.59	0.09-0.23	0.49-5.72	0.12-0.44	3.75-4.70
mean	8.98	1.16	7.67	0.15	1.93	0.30	4.30
Louisiana							
range	11.64-19.43	1.96-2.06	9.29-16.85	0.39-0.52	12.76-21.19	1.25-4.72	3.20-7.20
mean	15.45	2.02	12.98	0.45	16.93	2.37	5.44
North Carolina, South Carolina, Georgia							
range	34.40-108.70	8.57-21.12	22.86-83.22	2.97-4.36	0.60-8.01	1.17-4.98	6.99-10.20
mean	65.84	14.93	47.47	3.44	4.08	3.06	8.63

¹ Total DDT includes DDT, DDD, and DDE.

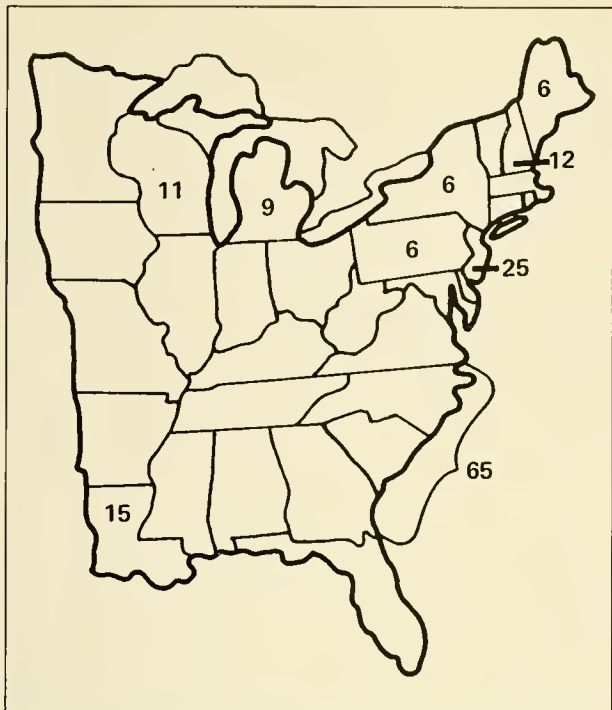


FIGURE 1.—Mean DDT residues (including DDT, DDD, and DDE) in woodcock wings (ppm, lipid weight basis)

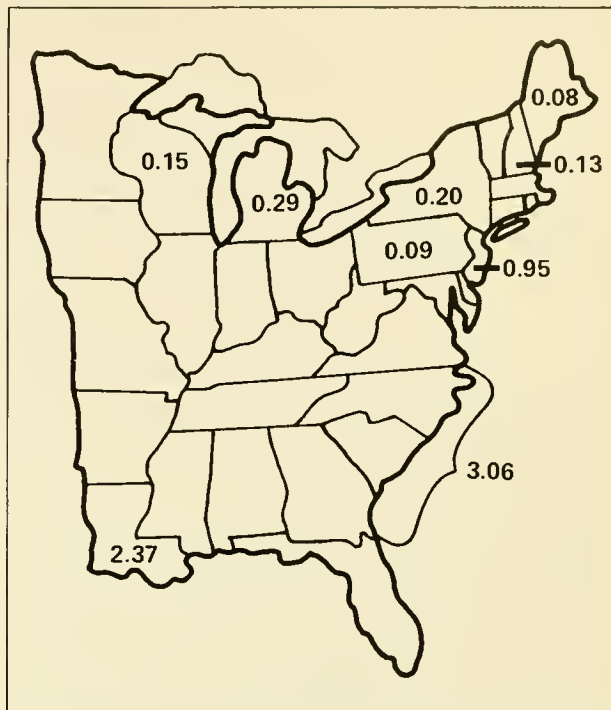


FIGURE 2.—Mean dieldrin residues in woodcock wings (ppm, lipid weight basis)

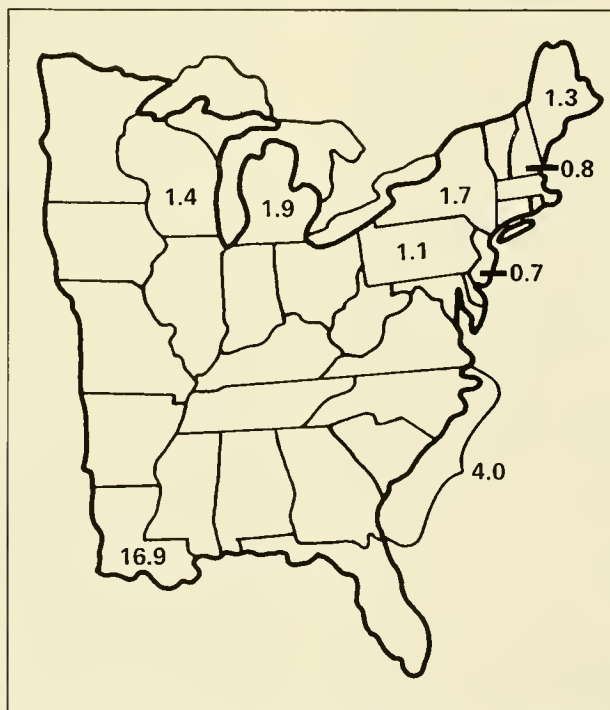


FIGURE 3.—Mean mirex residues in woodcock wings (ppm, lipid weight basis)

Mirex Incorporation in the Environment: Residues in Nontarget Organisms—1972

Syed M. Naqvi¹ and Armando A. de la Cruz²

ABSTRACT

A total of 78 samples representing 42 species of invertebrates, 3 species of fish, and 1 frog tadpole were randomly collected in 1972 from five sampling sites in Mississippi which had received varying degrees of mirex treatment. The samples were analyzed for mirex residues by gas chromatography. Residue levels in most samples were generally less than 1 ppm. Analyses of data provided observations on average residues (ppm) of pooled samples according to the following categories: (1) by type of organism—mollusks (0.15), fish (0.26), insects (0.29), crustaceans (0.44), and annelids (0.63); (2) by habitat—estuary (0.20), lake (0.27), grassland (0.28), creek (0.31), and pond (0.37); (3) by degree of mirex treatment at sampling site—localized treatment on fire ant mound (0.12), no treatment (0.19–0.20), aerial treatment in 1972 (0.33), and aerial treatment in 1968–70 (0.38); and (4) by trophic level—herbivore (0.23), carnivore (0.30), and omnivore (0.35). Mirex residues in animals collected from areas not treated with mirex suggest widespread movement of this pesticide in the environment, and the residues observed in the various trophic levels may be indicative of biological magnification.

Introduction

Mirex is a polycyclic chlorinated insecticide used in Mississippi and adjacent Southeastern States for control of the imported fire ant, *Solenopsis saevissima*—Forel

(2). In 1971, 4.5 million acres of open areas (non-woodland) were treated with fire ant bait by aerial application at the rate of 1.4 kg/hectare (1.25 lb of 4X bait/acre) which amounts to 4.2 g of the active ingredient of mirex per hectare. This was done two to three times during the year (C. C. Fancher, Mississippi Dept. of Agriculture, Jackson, Miss., *personal communication*). Mirex is the active ingredient of a bait designated as 4X, 2X, and 1X offered to fire ants. The 4X bait consists of 84.7% corncob grits, 15.0% soybean oil, and 0.3% mirex; 2X bait contains 0.15% mirex, and 1X bait, 0.1% mirex.

The presence of mirex residues in the tissues of a number of organisms reviewed by Alley (1) and our observations in the laboratory on the leaching of the toxic ingredient from the carrier bait and the ready uptake of this pesticide by aquatic invertebrates (4) prompted the routine analysis of residual mirex in nontarget organisms, particularly aquatic species. Although mirex is only slightly soluble in water, only about 1 ppb in fresh water and less in saline water (1), it could become potentially hazardous especially to detritivorous and neustonic organisms. No known metabolites of mirex have been reported in the literature. It is believed, however, that the toxicant is stored in quantities in animal tissues and is transported to all trophic levels in the food web. Theoretically, residue concentrations attain peak levels in the top consumers of the ecosystem. In this study, we are reporting the incorporation of mirex in the environment on the basis of residue levels in animals collected from various habitats in Mississippi.

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Methods and Procedures

SAMPLE COLLECTION

Animals were randomly collected from the following five sampling sites in Mississippi, four of which had received varying degrees of mirex treatment: Sampling site 1—Noxubee Wildlife Refuge (Noxubee County) had received no mirex treatment; Sampling site 2—Starkville (Oktibbeha County) had been treated by aerial application in 1968, 1969, and 1970—since then, treatment has been limited to hand application on individual fire ant mounds especially during the summer; Sampling site 3—Louisville-Noxapater site (Winston County) had been aerially treated during May and June 1972; Sampling site 4—St. Louis Bay Estuary (Hancock County) had received no direct mirex treatment although the county was sprayed aerially in 1971; Sampling site 5—Picayune (Pearl River County) had never been treated by airplane, but individual mound treatment has been in progress. All locations had been treated with 4X bait, except the Louisville-Noxapater site which had received 1X bait. This history of mirex spraying was provided by the Agricultural County Agents of each county.

The specimens on collection were cleaned of debris and mud when necessary, dipped quickly in acetone to remove external contamination, air-dried, wrapped in aluminum foil, and stored frozen when not immediately prepared for mirex analysis.

SAMPLE EXTRACTION AND CHROMATOGRAPHY

Pooled whole body samples numbering from 2 to 350 individuals, depending on size, and weighing from 1.32 to 3.90 g, wet-weight basis, were extracted for residue analyses by grinding in 20 g of a 1:8 mixture of washed sodium sulfate and ignited sea-sand. Grinding was enhanced by adding 15 ml of nanograde hexane to the mixture. Ground sample, solvent, and sand were placed in a 60-ml widemouth bottle, shaken for 30 minutes on an Eberbach laboratory shaker, and the solvent decanted into a collection beaker. This process was repeated twice, each time using 20 ml of nanograde hexane and shaking for 15 minutes. The extract was evaporated to dryness under a fume hood at $24^{\circ}\text{C} \pm 3^{\circ}\text{C}$. Prior to gas-liquid chromatographic analysis (GLC), the extracts were cleaned by using activated alumina. The method used was a modification of de Faubert Maunder (3) for organochlorine compounds.

In preparing the columns, the alumina and anhydrous sodium sulfate were washed twice with nanograde petroleum ether and finally with nanograde hexane. The alumina was activated by heating at 130°C for 12 hours and deactivated with 15% deionized distilled water. Five centimeters of deactivated alumina was placed into a

10 mm, OD X 30 cm fritted glass column. The adsorption material was layered with 1 cm of anhydrous sodium sulfate and reactivated by heating for 3 hours at 130°C . Whole-body extract was added to the column in 2-ml volumes in six successive washes. Columns were eluted with 100 ml of hexane by successive 5-ml volumes, and the elutions were evaporated to near dryness. Tests were conducted to determine the amount of toxicant recovered by the extraction and cleanup techniques. Uncontaminated, whole-body samples were spiked with known amounts of mirex; recovery was 90-99%.

Elution residues were diluted from 1 to 1,000 ml depending upon the concentration of mirex and analyzed on a Barber-Colman Model 5360 Pesticide Analyzer equipped with an electron-capture detector and a mixed column (6' X $1\frac{1}{4}$ ") consisting of 1.5% OV-17 and 1.95% QF-1 on 80/100 mesh Chromosorb — WHP. Standard injection techniques were employed consistently for all samples. Operating parameters for the analyzer were as follows: column temperature, 217°C ; nitrogen flow, 100 ml/min; and retention time for mirex, 27 minutes. Mirex quantification was done by peak height method according to the procedures of Gaul (4) and corrected for percent recovery. No confirmatory procedure for mirex was used.

Results and Discussion

Mirex residues in 78 samples representing 42 species of invertebrates, 3 species of fish, and 1 frog tadpole are tabulated by sampling location in Table 1. The residue levels showed a great deal of variation but were generally below 1 ppm. As can be seen in Table 1, concentrations ≥ 1 ppm were found in five samples, and > 2 ppm in only two samples. Higher concentrations have been detected in other species of nontarget organisms as reviewed by Alley (1), but these species were primarily small vertebrates occupying the upper level in the food chain. Analysis, by trophic level, of residue concentrations pooled from all the sampling locations revealed average values of 0.23, 0.31, and 0.35 ppm for herbivores, carnivores, and omnivores, respectively (Table 2). An investigation of biological magnification of mirex in the food chain as reflected by these values is being pursued in our continuing studies of mirex incorporation in the environment. Laboratory studies by Lowe *et al.* (7) and Ludke *et al.* (8) on the movement of mirex in simple food chains have shown that food items containing 0.5-1.0 ppm mirex caused up to 53% mortality in certain aquatic animals. If this were the case in nature, the residue levels detected in many of the organisms examined in this study are potentially poisonous to prospective consumer species which are highly sensitive to mirex.

The ability of animals to concentrate mirex and other chlorinated hydrocarbons varies widely (4,7,9). This was indicated by the levels observed when the samples were pooled by taxonomic categories as shown in Table 3. The highest average residue concentration was detected in the annelids (0.63 ppm), while the lowest average concentration was observed in the mollusks (0.15 ppm). Earlier studies by Gish (6) showed that oligochaetes were able to concentrate large quantities of chlorinated hydrocarbons, and among this order of annelids, tubificids were noted by Naqvi and Ferguson (9) to be extremely tolerant. In our annelid samples, leeches contained fairly high levels of mirex.

Residue averages, for animals pooled according to habitats and collection sites, are shown in Tables 4 and 5, respectively. Mirex residues in terrestrial organisms (e.g., grassland) are considered a result of the animals' direct contact with the pesticide, whereas mirex in samples from the four aquatic habitats is more a function of the leaching of toxicant from the bait carrier and subsequent incorporation into the organisms. Lowe *et al.* (7) have shown in field experiments that 66% of the active ingredient leached out of the fire ant bait after 9 months. Our preliminary data obtained from laboratory simulations of leaching indicated 19% leaching after 15 days. Furthermore, the physical parameters of the habitat influence the accumulation of the pesticide. As can be seen in Table 4, the residue levels in enclosed small ponds were slightly higher than in free flowing creeks; levels in creeks were higher than in larger bodies of lakes; and levels in lakes were higher than in the more contiguous bay-estuary.

The history of mirex application in the various sampling locations was discussed earlier in this report. As expected, organisms from locations which received aerial treatment in conjunction with the fire ant control program had higher concentration levels than organisms from other areas (Table 5). The presence of residues

in organisms from Bluff Lake (Noxubee County) and samples from St. Louis Bay Estuary (Hancock County) which did not receive any direct mirex treatment is evidence of the widespread movement of this compound in the environment.

See Appendix for chemical name of mirex.

Acknowledgment

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TABLE 1.—Mirex residues in nontarget organisms collected at five sites in Mississippi, 1972

SPECIES COLLECTED AND HABITAT		BASIC FOOD HABIT	MIREX RESIDUES IN PPM ¹ , WET- WEIGHT BASIS
SCIENTIFIC NAME	COMMON NAME		
BLUFF LAKE in Noxubee Wildlife Refuge (Noxubee Co., Miss.) ²			
Lake:			
<i>Neotettix proavus</i> (Orthoptera: Insecta)	Grasshopper	Herbivore	0.10
<i>Dichromorpha viridis</i> (Orthoptera: Insecta)	Grasshopper	Herbivore	0.15
<i>Gambusia affinis</i> (Poeciliidae: Pisces)	Mosquitofish	Carnivore	0.07
<i>Lepomis cyanellus</i> (Centrarchidae: Pisces)	Green sunfish	Carnivore	0.12
<i>Belostoma fluminea</i> (Hemiptera: Insecta)	Giant water bug	Carnivore	0.15
<i>Lepomis cyanellus</i> (Centrarchidae: Pisces)	Green sunfish	Carnivore	0.38
<i>Dolomedes sexpunctatus</i> (Pisauridae: Arachnida)	Spider	Carnivore	0.78
<i>Campeloma subdatum</i> (Gastropoda: Mollusca)	Snail	Omnivore	0.04
<i>Palaemonetes kadiakensis</i> (Decapoda: Crustacea)	Freshwater shrimp	Omnivore	0.06
<i>Palaemonetes kadiakensis</i> (Decapoda: Crustacea)	Freshwater shrimp	Omnivore	0.09
Total number of samples: 10 Average residue level: 0.19 ppm			
STARKVILLE AREA (Oktibbeha Co., Miss.) ³			
Pond:			
<i>Neohydrophilus castus</i> (Coleoptera: Insecta)	Water scavenger beetle	Herbivore	0.00
<i>Tropisternus</i> sp. (Coleoptera: Insecta)	Water scavenger beetle	Herbivore	0.36
<i>Sigara alternata</i> (Hemiptera: Insecta)	Water boatmen	Carnivore	0.03
<i>Belostoma fluminea</i> (Hemiptera: Insecta)	Giant water bug	Carnivore	0.09
<i>Notonecta undulata</i> (Hemiptera: Insecta)	Back swimmer	Carnivore	0.13
<i>Belostoma fluminea</i> (Hemiptera: Insecta)	Giant water bug	Carnivore	0.09
<i>Neoperla clymene</i> naiad (Odonata: Insecta)	Dragonfly	Carnivore	0.14
<i>Erpobdella punctata</i> (Hirudinea: Annelida)	Leech	Carnivore	0.24
<i>Macromia magnifica</i> naiad (Odonata: Insecta)	Dragonfly	Carnivore	0.31
<i>Placobdella rugosa</i> (Hirudinea: Annelida)	Leech	Carnivore	0.40
<i>Macromia</i> sp. naiad (Odonata: Insecta)	Dragonfly	Carnivore	1.92
<i>Orconectes lancifer</i> (Decapoda: Crustacea)	Crayfish	Omnivore	0.00

TABLE 1.—Mirex residues in nontarget organisms collected at five sites in Mississippi, 1972—Continued

SPECIES COLLECTED AND HABITAT		BASIC FOOD HABIT	MIREX RESIDUES IN PPM ¹ , WET- WEIGHT BASIS
SCIENTIFIC NAME	COMMON NAME		
STARKVILLE AREA (Oktibbeha Co., Miss.) ³ —Continued			
<i>Physa gyrina</i> (Gastropoda: Mollusca)	Snail	Omnivore	0.03
<i>Physa gyrina</i> (Gastropoda: Mollusca)	Snail	Omnivore	0.03
<i>Limnodrilus udekemianus</i> (Oligochaeta: Annelida)	Aquatic earthworm	Omnivore	0.13
<i>Physa gyrina</i> (Gastropoda: Mollusca)	Snail	Omnivore	0.26
<i>Procambarus hayi</i> (Decapoda: Crustacea)	Crayfish	Omnivore	0.26
<i>Palaemonetes kadiakensis</i> (Decapoda: Crustacea)	Freshwater shrimp	Omnivore	1.08
<i>Orconectes lancifer</i> (Decapoda: Crustacea)	Crayfish	Omnivore	2.09
Lake:			
<i>Halplus triopsts</i> (Coleoptera: Insecta)	Crawling water beetle	Herbivore	0.00
<i>Helocordulia uhleria</i> naiad (Odonata: Insecta)	Dragonfly	Carnivore	0.02
<i>Gerris remigis</i> (Hemiptera: Insecta)	Water strider	Carnivore	0.03
<i>Macromia</i> sp. naiad (Odonata: Insecta)	Dragonfly	Carnivore	0.67
<i>Hyallela azteca</i> (Amphipoda: Crustacea)	Sideswimmer	Omnivore	1.33
Grassland:			
<i>Dichromorpha viridis</i> (Orthoptera: Insecta)	Grasshopper	Herbivore	0.10
<i>Neotettix proavus</i> (Orthoptera: Insecta)	Grasshopper	Herbivore	0.24
<i>Neotettix proavus</i> (Orthoptera: Insecta)	Grasshopper	Herbivore	0.26
<i>Conocephalus</i> sp. (Orthoptera: Insecta)	Grasshopper	Herbivore	0.30
<i>Dichromorpha viridis</i> (Orthoptera: Insecta)	Grasshopper	Herbivore	0.70
<i>Mimetes puritans</i> (Mimetidae: Arachnida)	Spider	Carnivore	0.29
Total number of samples: 30 Average residue level: 0.38 ppm			
LOUISVILLE AND NOXAPATER AREAS (Winston Co., Miss.) ⁴			
Creek:			
<i>Peltodytes</i> sp. (Coleoptera: Insecta)	Crawling water beetle	Herbivore	0.12
<i>Rana grylio</i> tadpole (Anura: Amphibia)	Frog	Herbivore	0.12
<i>Hexagonea bilineata</i> larva (Ephemeroptera: Insecta)	Mayfly	Herbivore	0.90

TABLE 1.—Mirex residues in nontarget organisms collected at five sites in Mississippi, 1972—Continued

SPECIES COLLECTED AND HABITAT		BASIC FOOD HABIT	MIREX RESIDUES IN PPM ¹ , WET- WEIGHT BASIS
SCIENTIFIC NAME	COMMON NAME		
LOUISVILLE AND NOXAPATER AREAS (Winston Co., Miss.) ←Continued			
<i>Progomphus obscurus</i> naiad (Odonata: Insecta)	Dragonfly	Carnivore	0.00
<i>Lethocercus americanus</i> (Hemiptera: Insecta)	Giant water bug	Carnivore	0.04
<i>Progomphus obscurus</i> naiad (Odonata: Insecta)	Dragonfly	Carnivore	0.04
<i>Lethocercus americanus</i> (Hemiptera: Insecta)	Giant water bug	Carnivore	0.09
<i>Helocordulia uhleri</i> naiad (Odonata: Insecta)	Dragonfly	Carnivore	0.12
<i>Plathemis lydia</i> naiad (Odonata: Insecta)	Dragonfly	Carnivore	0.20
<i>Lethocercus americanus</i> (Hemiptera: Insecta)	Giant water bug	Carnivore	0.23
<i>Erpobdella punctata</i> (Hirudinea: Annelida)	Leech	Carnivore	1.76
<i>Palaemonetes kadiakensis</i> (Decapoda: Crustacea)	Freshwater shrimp	Omnivore	0.02
<i>Physa gyrina</i> (Gastropoda: Mollusca)	Snail	Omnivore	0.03
<i>Orconectes mississippiensis</i> (Decapoda: Crustacea)	Crayfish	Omnivore	0.10
<i>Eupera singleyi</i> (Pelecypoda: Mollusca)	Clam	Omnivore	0.15
<i>Gammarus limneus</i> (Amphipoda: Crustacea)	Sideswimmer	Omnivore	0.42
<i>Rhamphocorixa acuminata</i> (Hemiptera: Insecta)	Water boatmen	Omnivore	2.01
Pond:			
<i>Haliplus triopsis</i> (Coleoptera: Insecta)	Crawling water beetle	Herbivore	0.05
<i>Enallagma</i> sp. naiad (Zygoptera: Insecta)	Damselfly	Carnivore	0.07
<i>Buceona elegans</i> (Hemiptera: Insecta)	Backswimmer	Carnivore	0.10
<i>Lepomis cyanellus</i> (Centrarchidae: Pisces)	Green sunfish	Carnivore	0.17
<i>Buceona elegans</i> (Hemiptera: Insecta)	Backswimmer	Carnivore	0.22
<i>Macromia</i> sp. naiad (Odonata: Insecta)	Dragonfly	Carnivore	0.26
<i>Gambusia affinis</i> (Poeciliidae: Pisces)	Mosquitofish	Carnivore	1.00
Grassland:			
<i>Dichromorpha viridis</i> (Orthoptera: Insecta)	Grasshopper	Herbivore	0.10

Total number of samples: 25

Average residue level: 0.33 ppm

TABLE 1.—Mirex residues in nontarget organisms collected at five sites in Mississippi, 1972—Continued

SPECIES COLLECTED AND HABITAT		BASIC FOOD HABIT	MIREX RESIDUES IN PPM ¹ , WET- WEIGHT BASIS
SCIENTIFIC NAME	COMMON NAME		
PICAYUNE AREA (Pearl River Co., Miss.) ⁵			
Creek:			
<i>Lepomis cyanellus</i> (Centrarchidae: Pisces)	Green sunfish	Carnivore	0.03
<i>Gambusia affinis</i> (Poeciliidae: Pisces)	Mosquitofish	Carnivore	0.12
<i>Dineutes americanus</i> (Coleoptera: Insecta)	Whirligig beetle	Carnivore	0.22
<i>Noturus funebris</i> (Ictaluridae: Pisces)	Catfish	Carnivore	0.32
<i>Palaemonetes</i> sp. (Decapoda: Crustacea)	Shrimp	Omnivore	0.00
<i>Palaemonetes</i> sp. (Decapoda: Crustacea)	Shrimp	Omnivore	0.02
Total number of samples: 6 Average residue level: 0.12 ppm			
ST. LOUIS BAY ESTUARY (Hancock Co., Miss.) ⁶			
Estuary:			
<i>Crassostrea virginica</i> (Pelecypoda: Mollusca)	Oyster	Omnivore	0.07
<i>Crassostrea virginica</i> (Pelecypoda: Mollusca)	Oyster	Omnivore	0.11
<i>Marginella apicina</i> (Gastropoda: Mollusca)	Snail	Omnivore	0.19
<i>Polymesoda caroliniana</i> (Pelecypoda: Mollusca)	Clam	Omnivore	0.19
<i>Uca</i> sp. (Decapoda: Crustacea)	Crab	Omnivore	0.21
<i>Crassostrea virginica</i> (Pelecypoda: Mollusca)	Oyster	Omnivore	0.24
<i>Littorina anguilifera</i> (Gastropoda: Mollusca)	Snail	Omnivore	0.41
Total number of samples: 7 Average residue level: 0.20 ppm			

¹ Residue values represent single analysis of a pooled sample numbering from 2 to 350 individuals, depending on size, and weighing from 1.32 to 3.90 g.

² This area had not received any mirex treatment of any kind.

³ This area received the prescribed aerial treatment of mirex in 1968, 1969, and 1970.

⁴ These areas were treated with the prescribed dosage of mirex in summer 1972.

⁵ This area has never been aerially treated with mirex, but individual mound treatment has been initiated.

⁶ This area had never received mirex treatment directly, but Hancock County was sprayed aerially in 1971.

TABLE 2.—*Mirex residues in organisms grouped according to trophic levels*

TROPIC LEVEL	NUMBER OF SAMPLES	MIREX RESIDUES IN PPM, WET-WEIGHT BASIS		
		RANGE	MEDIAN	MEAN
Herbivores	15	0 - 0.90	0.12	0.23
Carnivores	36	0 - 1.92	0.15	0.30
Omnivores	27	0 - 2.09	0.13	0.35

TABLE 3.—*Mirex residues in organisms grouped according to taxonomic categories*

TROPIC LEVEL	NUMBER OF SAMPLES	MIREX RESIDUES IN PPM, WET-WEIGHT BASIS		
		RANGE	MEDIAN	MEAN
Mollusca	12	0.03 - 0.41	0.13	0.15
Pisces and Amphibia	9	0.03 - 1.00	0.12	0.26
Insecta and Arachnida	40	0 - 2.01	0.14	0.29
Crustacea	13	0 - 2.09	0.10	0.44
Annelida	4	0.13 - 1.76	0.32	0.63

TABLE 4.—*Mirex residues in organisms grouped according to habitat*

HABITAT	NUMBER OF POOLED SAMPLES	MIREX RESIDUES IN PPM, WET-WEIGHT BASIS		
		RANGE	MEDIAN	MEAN
Estuary	7	0.07 - 0.41	0.19	0.20
Lake	15	0 - 1.33	0.10	0.27
Grassland	7	0.10 - 0.70	0.26	0.28
Creek	23	0 - 2.01	0.12	0.31
Pond	26	0 - 2.09	0.16	0.37

TABLE 5.—*Mirex residues in organisms grouped according to sampling sites*

SAMPLING SITES ¹	NUMBER OF SAMPLES	MIREX RESIDUES IN PPM, WET-WEIGHT BASIS		
		RANGE	MEDIAN	MEAN
Picayune, Pearl River County	6	0 - 0.32	0.08	0.12
St. Louis Bay Estuary, Hancock County	7	0.07 - 0.41	0.19	0.19
Noxubee Refuge, Noxubee County	10	0.04 - 0.78	0.11	0.19
Louisville and Noxapater, Winston County	25	0 - 2.01	0.12	0.33
Starkville, Oktibbeha County	30	0 - 2.09	0.24	0.38

¹ Picayune received individual mound treatment; St. Louis Bay Estuary and Noxubee Refuge did not receive any form of mirex application; Louisville-Noxapater was aerially treated in 1972; and Starkville received several aerial treatments since 1968.

Accumulation of Mirex Residues in Selected Organisms After an Aerial Treatment, Mississippi—1971-72

James L. Wolfe¹ and B. R. Norment²

ABSTRACT

Mirex residues were monitored in selected organisms in Mattubby Creek Watershed, Monroe County, Miss., for approximately 1 year following an aerial application of 1X mirex bait (0.85 g/acre). Although there was an increase in residues in crayfish, levels remained relatively low in invertebrates as compared to those previously reported. Fishes from streams in the treated areas showed an increase in residues of about 2 to 20 times over controls taken from an adjacent watershed. These levels in stream fishes remained relatively constant for 7 months after treatment, when the last sample was taken. Residue levels in mammals were obviously stratified according to food habits. Strict herbivores had the lowest levels, omnivores intermediate levels, and insectivore-carnivores the highest levels. Generally, the residues detected were lower than those previously reported and presumably reflected the lower rate of mirex application (1X rate versus the normal 4X or 2X rate). The fact that the compound was concentrated by certain nontarget organisms even at this low rate of application and stratified by trophic level is worthy of note.

Introduction

Environmental pollution is one of the most pertinent and controversial issues today. Chlorinated hydrocarbon pesticides are some of the most frequently mentioned offenders, chiefly because of their long residual properties and their accumulation by plants and animals in the food chain of man (9). Currently, a stable chlorinated hydrocarbon pesticide, mirex, is being used in the

Southern United States in efforts to control the spotted imported fire ant, *Solenopsis saevissima richteri* (Forel), and the imported fire ant, *Solenopsis invicta* (Buren); and a control program is being implemented to treat large areas in Mississippi with mirex bait. In 1971-72, this study of selected aquatic and terrestrial vertebrate and invertebrate populations from the Mattubby Creek Watershed, Monroe County, Miss., was initiated to determine the extent of mirex residues in a freshwater ecosystem following an aerial application of mirex.

Val Valin *et al.* (10) reported that at the recommended rate, the concentration of mirex residues in soil, water, and vegetation was relatively constant for over 300 days. Other studies (1,3,4) have shown that the acute toxicity of mirex to birds and mammals is extremely low. Similarly, low toxicity to fishes has been recorded (2,5). Chronic effects of sublethal levels have not been investigated in any detail and these may be important because the chemical is persistent. Its high toxicity to nontarget arthropods, especially crustacea, has been demonstrated in the laboratory by Lowe *et al.* (5) and Ludke *et al.* (6).

Study Area

The Mattubby Creek Watershed of Monroe County, Miss., was selected for study. This area was last treated with mirex in 1968 as was most of east-central Mississippi. After pretreatment sampling in the summer and early fall of 1971, the area received an aerial application of 1X mirex bait on Sept. 15, 1971.

Organisms for residue analysis were collected from permanent and temporary ponds, streams, and terrestrial

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habitats. A 1-acre permanent pond east of Lake Monroe (about 7 miles north of Aberdeen) was used extensively for collecting and exposing caged catfish. Two temporary pools approximately 3 miles south of this pond were also used for collecting. Streams sampled in the treatment area included Mattubby Creek, Wolf Creek, and Cedar Creek. Control streams in an adjacent watershed which was not subjected to the 1971 treatment were Kettle Creek, James Creek, and Nichols Creek. A number of terrestrial areas, primarily old fields in early successional stages, in a quadrangle 7 miles north and west of Aberdeen were sampled.

Sample Collection

INVERTEBRATES

Invertebrates collected consisted of crayfish (*Orconectes* sp.), mosquito larvae (mixed species), and field crickets (*Acheta assimilis*). Pre- and post-treatment samples of all three organisms were obtained and analyzed at approximately regular intervals over a 12-month period. Crayfish were collected from the permanent and the two temporary ponds; mosquito larvae were collected from temporary pools. The cricket samples all came from a single field locality.

POND FISHES

Two species were analyzed from the permanent pond. Channel catfish, *Ictalurus punctatus* (80, 3-inch fingerlings in a wire cage and 2,000 released as 1-inch fingerlings), were placed in the pond. Naturally occurring green sunfish (*Lepomis cyanellus*) were seined from the pond. Fish samples were analyzed at the same intervals as were the invertebrates.

STREAM FISHES

A total of 10 species of stream fishes were analyzed for mirex residues (Table 2). No pretreatment samples were taken, but concurrent samples were taken from streams in an adjacent, untreated (since 1968) drainage area. Collections for analysis were made by seining in November 1971 and April 1972.

MAMMALS

Seven species of small mammals were captured and analyzed. These included the cotton rat (*Sigmodon hispidus*), the rice rat (*Oryzomys palustris*), the harvest mouse (*Reithrodontomys humulis*), the pine mouse (*Pitymys pinetorum*), the wood mouse (*Peromyscus leucopus*), the house mouse (*Mus musculus*), and the short-tailed shrew (*Blarina brevicauda*). Collections were made in September (prior to treatment), December 1971, and March and May 1972. Animals were captured alive in Sherman traps.

Sample Preparation and Analytical Procedure

Invertebrates and fishes were packed in ice in the field and frozen on arrival at the laboratory. Mammals were brought in alive and then frozen. Residues given are on a whole-body basis unless otherwise stated. In the case of invertebrates and fishes, several specimens were pooled and homogenized to make a sample. For the mammals, one specimen equals one sample. All analyses were performed by the Mississippi State Chemical Laboratory using standard FDA procedures for biological materials (7). The one departure from the referenced procedure was that two extractions rather than the recommended one were used. Previous work on fish and bird samples by the laboratory involving exhaustive (6 to 9) extractions indicated that two extractions removed from 90 to 95% of the mirex from the sample. A Barber-Coleman (Model 5360) Pesticide Analyzer with a Ni⁶³ detector was used for the analyses. A mixed column, OV-210/OV-1, was used for quantitation and a DEGS column for confirmation. The sensitivity of the method was approximately 0.01 ppm.

Results and Discussion

Residue data for invertebrates are given in Table 1. In crayfish, no residues were detected in 2 pretreatment samples, but residues averaging 0.07 ppm were detected in all 8 posttreatment samples. In insect samples, there was no clear indication of an increase in residue levels during the posttreatment period. Levels for both crickets and crayfish were lower than those reported after one treatment by Markin *et al.* (8).

Samples of green sunfish taken from the permanent pond in July and August each contained 0.02 ppm mirex. A sample taken on September 22, just after the aerial application, had 0.01 ppm. Samples collected from November through February did not contain detectable mirex levels; no sunfish were found from April through July. A pretreatment catfish sample taken in early September did not contain mirex. Residues in three post-treatment samples of the caged *I. punctatus* fingerlings taken in September, October, and November ranged from 0.01 to 0.02 ppm. In January the caged fingerlings were gone (had apparently died and decomposed), and none were captured on six subsequent seining trips. On November 2, differences in residues were apparent in fish species taken from untreated and treated streams (Table 2). Mosquitofish (*Gambusia affinis*) from untreated streams averaged about 0.05 ppm, whereas those from treated areas averaged 0.54 ppm. While there was little difference in the top minnow samples from the two areas, residues in the Cyprinid, *Notropis bellus*, a pretty shiner, from the treated area were considerably higher than those from the untreated area (0.30 vs. 0.06 ppm). A redbfin pickerel (*Esox americanus americanus*), pre-

sumably a top carnivore, from the treated area had a residue of 0.53 ppm. None were collected from an untreated stream on this trip, but in April one collected from an untreated area had 0.02 ppm.

Five months later (April 26), the situation appeared relatively unchanged. Previously, the highest level detected, 0.92 ppm, was in a mosquitofish, a topwater form. In April the highest residues (0.97, 1.02 ppm) were found in Cyprinids (shiners), which are primarily mid-water forms. Typically, species or groups from treated areas showed increases in mirex residues of 2 to 10 times over those from untreated areas (Table 2).

A sample of small mammals (cotton rats, rice rats) collected in the Mattubby Creek watershed on Sept. 4, 1971, showed extremely low mirex residues. No residues were detected in cotton rats, while small amounts (0.01 ppm) were detected in rice rats. In December, residues of 0.02 were detected in house mice (Table 3). In March, cotton rats had residues as high as 0.02 ppm which was not a significant increase, but two species of mice (harvest mice and white-footed mice), which are similar to rice rats and house mice in food habits, had levels as high as 0.54 ppm. On this date the first collection of shrews was also made. Shrews, which eat primarily invertebrate animals and occasionally small vertebrates and from which the highest mirex residue of any mammal has been reported, Markin *et al.* (8), had residue levels as high as 1.89 ppm (Table 3). In Table 4 small mammals are classified as to eating habits and mirex residues detected. There is an obvious relationship between the small mammals' diet and mirex in the tissues. Levels for the white-footed mouse after the single treatment were about the same as those reported by Markin *et al.* (8) for the closely related cotton mouse after three treatments. Markin *et al.* (8) detected much higher levels in shrews after 1 or 2 treatments, up to 41.67 ppm, than in this study.

One group of samples was run to try to establish the relationship between the standard whole-body samples and mirex levels in the various tissues. These results are given in Table 5. From these data it appears that whole-body residues approximate muscle residues, with brain levels being slightly lower and liver levels being much (2 to 20 times) higher.

Data revealed that mirex levels in several invertebrate and vertebrate samples taken during the posttreatment period were higher than those taken during the pretreatment period and from untreated areas. The presence of mirex in pretreatment samples indicated the well-known stability of the compound when applied to the environment since the last known mirex application in Monroe County had been in 1968. Also revealed was

the possibility that mirex levels were more localized and that the residues detected in the specimens during the posttreatment period were due to the September 1971 application.

The mirex residues found in these specimens were lower than levels reported for the same species in the study by Markin *et al.* (8). These differences may possibly reflect the rate of mirex applications; our study area received a 1X (0.85 g/acre) application as opposed to the normal 4X (3.4 g/acre) or 2X (1.7 g/acre) rates. The fact that these various nontarget organisms accumulated mirex residues at one-quarter the recommended rate points to the ever-increasing importance of distribution and movement in the ecosystem. Previously published work (5,8,11) suggests the possibility of stratification and biological magnification of mirex in aquatic and terrestrial ecosystems. Our work further supports this hypothesis.

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See Appendix for chemical name of mirex.

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TABLE 1.—*Mirex residues in aquatic and terrestrial invertebrates before and after treatment of the Mattubby Creek Watershed in Monroe County, Miss.—1971-72*

SPECIES	RESIDUES IN PPM BY COLLECTION DATES												
	PRETREATMENT			POSTTREATMENT									
	7/23	8/25	9/11	9/22	10/29	11/2	1/12	1/25	2/14	4/20	5/10	6/7	7/5
Crayfish (<i>Orconectes</i> sp.)		0.00	0.00			0.04	0.06	0.16	¹ 0.09	0.05	0.04	0.05	0.04
Mosquito larvae		0.00	0.00		0.00			0.00	0.00	0.03	0.02	0.00	0.00
Field cricket (<i>Acheta assimilis</i>)		0.00	0.00	0.03	0.00	0.02	0.01		0.00		0.00	0.02	0.03

NOTE: Blank = no sample collected; several specimens were pooled and homogenized to make a sample; residues reported are on a whole-body basis unless otherwise indicated.

¹ Average of three samples (0.02 - 0.22 ppm).

TABLE 2.—*Mirex residues in stream fishes before and after treatment of the Mattubby Creek Watershed in Monroe County, Miss.—1971-72*

SPECIES	RESIDUES IN PPM											
	UNTREATED STREAMS						TREATED STREAMS					
	NOVEMBER 1971			APRIL 1972			NOVEMBER 1971			APRIL 1972		
	NUMBER IN SAMPLE	MEAN	RANGE	NUMBER IN SAMPLE	MEAN	RANGE	NUMBER IN SAMPLE	MEAN	RANGE	NUMBER IN SAMPLE	MEAN	RANGE
Redfin pickerel (<i>Esox americanus americanus</i>)				1	0.02		1	0.53				
Pretty shiner (<i>Notropis bellus</i>)	1	0.06					2	0.29	(0.04- 0.55)	3	0.41	(0.10- 0.97)
Blacktail shiner (<i>N. venustus</i>)				1	0.03					1	0.22	
Bluntnose minnow (<i>Pimephales notatus</i>)										2	0.76	(0.51- 1.02)
Top minnow (<i>Fundulus</i> sp.)	1	0.01		1	0.02		1	0.03		2	0.08	(0.07- 0.10)
Mosquitofish (<i>Gambusia affinis</i>)	3	0.04	(0.00- 0.06)	1	0.04		2	0.53	(0.15- 0.92)	5	0.15	(0.04 0.26)
Green sunfish (<i>Lepomis cyanellus</i>)	1	0.00					2	0.10	(0.05- 0.15)	1	0.03	
Bluegill (<i>L. Macrochirus</i>)	3	0.02	(0.01- 0.04)	1	0.02		2	0.01	(0.01- 0.02)	1	0.11	
Largemouth bass (<i>Micropterus salmoides</i>)				1	0.05					1	0.18	
Darter (<i>Etheostoma</i> sp.)	1	0.02					2	0.04				

NOTE: Each sample was taken from a different stream; Blank = no sample collected.

TABLE 3.—Mirex residues in small mammals collected in Monroe County, Miss., Sept. 4, 1971-March 22 and May, 1972

SPECIES	RESIDUES IN PPM								
	SEPTEMBER 4			DECEMBER 21			MARCH 22 AND MAY		
	NUMBER IN SAMPLE	MEAN	RANGE	NUMBER IN SAMPLE	MEAN	RANGE	NUMBER IN SAMPLE	MEAN	RANGE
Cotton rat (<i>Sigmodon hispidus</i>)	6	0.00		2	0.00		2	0.01	(0.01-0.02)
Rice rat (<i>Oryzomys palustris</i>)	2	0.00	(0.00-0.01)	3	0.00	(0.00-0.01)			
House mouse (<i>Mus musculus</i>)				3	0.01	(0.00-0.02)			
Harvest mouse (<i>Reithrodontomys humulis</i>)							1	0.54	
Wood mouse (<i>Peromyscus leucopus</i>)							5	0.15	(0.04-0.21)
Pine mouse (<i>Pitymys pinetorum</i>)							1	0.02	
Short-tailed shrew (<i>Blarina brevicauda</i>)							5	1.00	(0.10-1.89)

NOTE: Blank = no sample collected.

TABLE 4.—Relationship of diet to mirex residues in small mammals collected in treated area of Monroe County, Miss. 1972

TROPHIC LEVEL	MIREX RESIDUES IN PPM ¹
Herbivores (Feed principally on plants— <i>Sigmodon</i> , <i>Pitymys</i>)	0.01
Omnivores (Feed principally on plant products and invertebrates— <i>Oryzomys</i> , <i>Peromyscus</i> , <i>Reithrodontomys</i>)	0.21
Carnivores (Feed principally on invertebrates with some vertebrate material— <i>Blarina</i>)	1.00

¹ Data taken from specimens collected on March 22, 1972.

TABLE 5.—A comparison of whole body and tissue levels of three species of small mammals collected from the treated area of Monroe County, Miss., March 17-19, 1972

SPECIES	SAMPLE No.	RESIDUES IN PPM			
		WHOLE BODY	MUSCLE	BRAIN	LIVER
<i>Sigmodon</i>	1	0.00			
	2	0.00			
	3		0.00	0.00	0.04
	4		0.00	0.00	0.01
	5		0.00	0.00	0.01
	6		0.01	0.00	0.02
	7		0.00	0.00	0.03
<i>Oryzomys</i>	1	0.24			
	2		0.09	0.17	1.75
	3		0.20	0.16	2.45
<i>Blarina</i>	1	0.26			
	2		0.25	0.10	0.42

APPENDIX

Chemical Names of Compounds Discussed in This Issue

BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
DDE	1,1-dichloro-2,2-bis(<i>p</i> -chlorophenyl) ethylene
DDT	1,1,1-trichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane, technical DDT consists of a mixture of the <i>p,p'</i> -isomer and the <i>o,p'</i> -isomer (in a ratio of about 3 or 4 to 1)
DIELDRIN	Not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4- <i>endo-exo</i> -5,8-dimethano=naphthalene
MIREX	dodecachlorooctahydro-1,3,4-metheno-2 <i>H</i> -cyclobuta[<i>cd</i>]pentalene
POLYCHLORINATED BIPHENYLS (PCB's)	Mixtures of chlorinated biphenyl compounds having various percentages of chlorination
TDE (DDD) (including its isomers and dehydrochlorina- tion products)	1,1-dichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane; technical TDE contains some <i>o,p'</i> -isomer also

Information for Contributors

The PESTICIDES MONITORING JOURNAL welcomes from all sources qualified data and interpretive information which contribute to the understanding and evaluation of pesticides and their residues in relation to man and his environment.

The publication is distributed principally to scientists and technicians associated with pesticide monitoring, research, and other programs concerned with the fate of pesticides following their application. Additional circulation is maintained for persons with related interests, notably those in the agricultural, chemical manufacturing, and food processing industries; medical and public health workers; and conservationists. Authors are responsible for the accuracy and validity of their data and interpretations, including tables, charts, and references. Accuracy, reliability, and limitations of the sampling and analytical methods employed must be clearly demonstrated through the use of appropriate procedures, such as recovery experiments at appropriate levels, confirmatory tests, internal standards, and inter-laboratory checks. The procedure employed should be referenced or outlined in brief form, and crucial points or modifications should be noted. Check or control samples should be employed where possible, and the sensitivity of the method should be given, particularly when very low levels of pesticides are being reported. Specific note should be made regarding correction of data for percent recoveries.

- Preparation of manuscripts should be in conformance to the CBE STYLE MANUAL, 3d ed. Council of Biological Editors, Committee on Form and Style, American Institute of Biological Sciences, Washington, D. C. and/or the STYLE MANUAL of The United States Government Printing Office.
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- All copy, including tables and references, should be double spaced, and all pages should be numbered. The first page of the manuscript must contain authors' full names listed under the title, with affiliations, and addresses footnoted below.
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a notation in text to show where they should be inserted.

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- Photographs should be made on glossy paper. Details should be clear, but size is not important.
- The "number system" should be used for literature citations in the text. List references in the order in which they are cited in the text, giving name of author/s/, year, full title of article, exact name of periodical, volume, and inclusive pages.

The Journal also welcomes "brief" papers reporting monitoring data of a preliminary nature or studies of limited scope. A section entitled *Briefs* will be included, as necessary, to provide space for papers of this type to present timely and informative data. These papers must be limited in length to two journal pages (850 words) and should conform to the format for regular papers accepted by the Journal.

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The WORKING GROUP is comprised of representatives of the U. S. Departments of Agriculture; Commerce; Defense; the Interior; Health, Education, and Welfare; State; Transportation; and Labor; and the Environmental Protection Agency.

The pesticide MONITORING PANEL consists of representatives of the Agricultural Research Service, Consumer and Marketing Service, Extension Service, Forest Service, Department of Defense, Fish and Wildlife Service, Geological Survey, Food and Drug Administration, Environmental Protection Agency, National Marine Fisheries Service, National Science Foundation, and Tennessee Valley Authority.

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Pesticide monitoring activities of the Federal Government, particularly in those agencies represented on the pesticide MONITORING PANEL which participate in operation of the national pesticides monitoring network, are expected to be the principal sources of data and interpretive articles. However, pertinent data *in summarized form*, together with interpretive discussions, are invited from both Federal and non-Federal sources, including those associated with State and community monitoring programs, universities, hospitals, and nongovernmental research institutions, both domestic and foreign. Results of studies in which monitoring data play a major or minor role or serve as support for research investigation also are welcome; however, the *Journal* is not intended as a primary medium for the publication of basic research. Manuscripts received for publication are reviewed by an Editorial Advisory Board established by the MONITORING PANEL. Authors are given the benefit of review comments prior to publication.

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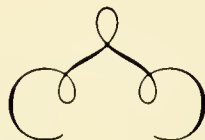
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EDITORIAL



A Tribute to Sylvia O'Rear and Her Staff

Self-praise is seldom viewed with admiration. For a special reason, however, the Editorial Advisory Board feels it is appropriate to boast momentarily about the success of the *Pesticides Monitoring Journal*. Specifically, the reason is to pay tribute to Sylvia O'Rear, former *PMJ* Editorial Manager, and to members of her staff, particularly Lynn Herring and Priscilla Holman, technical editors.

As a result of recent organizational changes within the U.S. Environmental Protection Agency, the function of the Editorial Manager has been transferred from Chamblee, Georgia, to Washington, D.C. However, Mrs. O'Rear and her staff were unable to come to Washington. Because these individuals' contributions to the *Journal* have been outstanding, the Monitoring Panel and the Editorial Advisory Board unanimously decided to dedicate this issue of the *Journal* to them.

Perhaps the most fitting tribute that could be given this staff is a statement on the accomplishments of the *Journal*. Although the publication is relatively young, it has in a little over six years achieved national and international recognition as evidenced by the large number of worldwide subscribers. In addition, with each new issue of the *Journal* it becomes more evident that non-Federal sources, as well as foreign sources, are providing an increasing number of papers for publication. We believe these facts are an indication that the *Journal* has become one of stature in its field and that Mrs. O'Rear and her staff played a major role in this accomplishment.

The Monitoring Panel and the Editorial Advisory Board recognize that the Editorial Staff has been instrumental in developing and maintaining the confidence of authors and of other editorial staffs. Since the *Journal's* initial publication in June 1967, the editors have attained a high level of competence in scientific editing. Their understanding of the pesticides field has been invaluable in evaluating each manuscript and determining the extent of editing required. In spite of extensive editing on many manuscripts, the record shows that Sylvia, Lynn, and Priscilla never offended a single author but, on the contrary, almost always received praise and gratitude for their efficient manner of editing. We are confident that our readers agree that the quality of the *Journal* exemplifies the efforts of the Editorial Staff in this area.

Above all, the personal dedication of this fine staff will be missed. The Monitoring Panel and the Editorial Advisory Board commend them for a job well done and extend our best wishes.

We would be remiss if we did not acknowledge that Paul Fuschini, the new Editorial Manager, and his staff fully recognize the importance of their new role. We wish to assure *PMJ* readers and contributing authors that the Editorial Advisory Board pledges its full support to the new Editorial Staff in helping insure that the *Journal* will continue to merit the respect of the scientific community.

John R. Wessel
Chairman
Editorial Advisory Board, *PMJ*

PESTICIDES IN PEOPLE

Organochlorine Pesticide and Polychlorinated Biphenyl Residues in Biopsied Human Adipose Tissue—Texas 1969-72

James E. Burns¹

ABSTRACT

Organochlorine pesticide residue levels were determined in 221 samples of human adipose tissue from elective surgery in 1969-72 in the lower Rio Grande Valley of Texas. Standard electron capture—gas-liquid chromatographic methods were used. The total DDT level was 23.18 ppm; the DDE level was 17.37, the highest yet reported for a general population. Dieldrin and β BHC levels were also high: 0.35 and 1.29 ppm, respectively. No decrease in storage levels during the study period was detected. There was no difference due to sex, but Mexican-Americans had significantly higher residues of DDE, p,p'-DDT, and dieldrin than did Anglo-Americans. Polychlorinated biphenyls were detected in 15 samples in 1971 but none were detected in the other 3 years.

Introduction

The presence of organochlorine pesticide residues in humans and other nontarget organisms as a reflection of high lipid solubility and unusual stability has generated much investigation. These studies have attempted to monitor temporal and geographic variations in residues (1-14). The effort included epidemiological investigations to define factors modifying residue levels and to detect alterations in morbidity and mortality (3, 7, 9, 15). This study reports organochlorine residues in adipose tissue of persons undergoing elective surgery in 1969-72 in the lower Rio Grande Valley of southeast Texas. The study area is a geographically isolated and well-defined semitropical agricultural region. It has been subjected to heavy pesticides usage, predominantly on cotton. Incidental to the investigation of pesticide residues has been the detection of polychlorinated biphenyls (PCB's). These compounds have been detected widely

in the environment and in human tissue (16, 17). This report also includes data on PCB's in the adipose samples analyzed for organochlorine residues.

Methods and Materials

A total of 302 samples of adipose tissue of approximately 5 g each were obtained from elective surgeries performed at one local hospital in 1969-72. The samples were frozen within 30 minutes of excision and stored in glass containers with foil-lined lids at -18°C until analysis. Demographic data were obtained by chart inspection; 81 samples were eliminated because of ambiguities, inconsistencies, insufficient lipid ($<30\%$), or nonlocal residence. It was not possible to determine a pesticide exposure history but none of the samples came from people with known unusual exposure. Extraction, cleanup, and analyses were performed in compliance with the methods prescribed for Community Pesticide Studies Laboratories (18). The extraction and cleanup procedures followed the modified Mills, Onley, and Gaither method (18, 19).

The concentrated 6% and 15% diethyl ether in petroleum ether fractions were injected into a Micro-Tek 220 gas chromatograph using a tritium foil electron-capture detector and a 6-ft-by- $\frac{1}{4}$ -in. outer-diameter glass column. The column contained one of two combinations: 1.5% OV-17 and 1.95% QF-1 on 100-120 mesh Chromosorb W (HP), or 4% SE-30 and 6% OV-210 on 80-100 mesh Chromosorb W (HP). Operating conditions were: column, 200°C ; inlet, 225°C ; detector, 205°C ; carrier gas, nitrogen; flow rate, 70 ml/min; parallel plate electron capture detector operated at DC voltage to cause $\frac{1}{2}$ full-scale recorder deflection for 50 pg aldrin; attenuation, 10 by 16; operating voltage, 22 v; total theoretical plates, > 3500 for each column.

¹ U.S. Public Health Service, assigned to Technical Services Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, 152 East Stenger, San Benito, Texas 78586.

Quantitation was by peak height and all residues were calculated on a total extractable lipid basis. When there was any question of peak identity, thin-layer chromatography (TLC) confirmation was obtained. All 6% fractions were scanned by electron-capture—gas-liquid chromatography (EC-GLC) and TLC for PCB's and quantitated visually by TLC using Aroclor 1260 as the reference standard.

Inter- and intralaboratory quality control samples and all standards were supplied by the Primate and Pesticides Effects Laboratory, Perrine, Florida. Quality control samples were stored and analyzed with each batch of study samples and the laboratory was in control for the entire study period.

Recovery studies for all residues except PCB's were consistently above 80% and usually above 90% during the study period; residues are reported in this paper without adjustment. Recovery studies were not performed for PCB's but the method is known to have a

precision of $\pm 50\%$ when Aroclor 1260 is the reference standard (18).

The limits of detectability for the residues reported are: 0.01 ppm for heptachlor epoxide, DDE, and dieldrin; 0.02 ppm for β BHC, *o,p'*-DDT, TDE, and *p,p'*-DDT; and 0.5 ppm for PCB's. Chemical names appear in the Appendix.

Results

Table 1 presents the mean level of the organochlorine residues found in adipose tissue of residents of the lower Rio Grande Valley of Texas for the study period 1969-72. Table 2 shows the percent occurrence of the same residues. In spite of the greatly decreased use of all these agents since 1968 and the abstinence, due to insect resistance, from DDT usage during the period, the level of these residues did not decrease. There are some apparently random, statistically significant variations but there are no trends.

TABLE 1.—Mean organochlorine pesticide residues in human adipose tissue from elective surgery—Texas, 1969-72

		RESIDUE: PPM (MEAN $\pm$ STANDARD DEVIATION) ¹							
	No.	DDT-R ²	DDE	<i>p,p'</i> -DDT	TDE	<i>o,p'</i> -DDT	DIELDRIN	HE	β BHC
Total	221	23.18 $\pm$ 15.36	17.37 $\pm$ 10.90	3.48 $\pm$ 2.54	.10 $\pm$.11	.24 $\pm$.28	.35 $\pm$.23	.11 $\pm$.11	1.23 $\pm$.95
Year									
1969	26	23.04 $\pm$ 17.50	16.50 $\pm$ 11.86	4.12 $\pm$ 2.56	.14 $\pm$.08	.34 $\pm$.23	.33 $\pm$.23	.14 $\pm$.09	.93 $\pm$.41
1970	68	21.03 $\pm$ 11.15	15.95 $\pm$ 8.04	3.07 $\pm$ 2.29	.09 $\pm$.12	.10 $\pm$.14 ³	.29 $\pm$.22	.08 $\pm$.07 ⁴	1.21 $\pm$.87
1971	88	22.32 $\pm$ 15.79	16.58 $\pm$ 11.26	3.44 $\pm$ 2.53	.08 $\pm$.09	.32 $\pm$.34	.36 $\pm$.21	.14 $\pm$.10 ⁵	1.34 $\pm$ 1.21
1972	39	29.29 $\pm$ 17.35	22.52 $\pm$ 12.10 ⁴	3.08 $\pm$ 2.75	.14 $\pm$.13	.25 $\pm$.27	.43 $\pm$.28	.14 $\pm$.13	1.20 $\pm$.66
Sex									
Female	122	23.04 $\pm$ 14.72	17.12 $\pm$ 10.33	3.62 $\pm$ 2.71	.10 $\pm$.11	.24 $\pm$.24	.35 $\pm$.25	.11 $\pm$.11	1.20 $\pm$ 1.10
Male	99	23.22 $\pm$ 16.03	17.55 $\pm$ 11.56	3.30 $\pm$ 2.30	.11 $\pm$.11	.25 $\pm$.33	.34 $\pm$.20	.11 $\pm$.10	1.26 $\pm$.72
Ethnic ³									
M.A.	151	25.75 $\pm$ 16.16	19.35 $\pm$ 11.73	3.84 $\pm$ 2.58	.10 $\pm$.12	.24 $\pm$.23	.38 $\pm$.22	.12 $\pm$.11	1.23 $\pm$ 1.04
Other	70	17.85 $\pm$ 10.98 ⁵	13.31 $\pm$ 7.33 ⁵	2.70 $\pm$ 2.28 ⁵	.08 $\pm$.08	.24 $\pm$.38	.27 $\pm$.18 ⁵	.11 $\pm$.10	1.17 $\pm$.73

¹ When $p > .01$, no notation appears; 4 values $\geq 10x$ the mean have been eliminated. All residues calculated on a total extractable lipid basis.

² DDT-R = DDT + 1.114 (DDE + TDE).

³ M.A. = Mexican-American (Spanish surname); other = Non-Spanish surname.

⁴ $p < .01$.

⁵ $p < .001$ (t-test).

TABLE 2.—Organochlorine pesticide residues in human adipose tissue from elective surgery—Texas, 1969-72

		PERCENT OCCURRENCE							
	No.	DDT-R ¹	DDE	<i>p,p'</i> -DDT	TDE	<i>o,p'</i> -DDT	DIELDRIN	HE	β BHC
Total	221	100	100	100	93	81	98	98	99
Year									
1969	26	100	100	100	96	93	89	89	93
1970	68	100	100	99	90	56	99	97	100
1971	88	100	100	100	84	88	100	100	100
1972	39	100	100	100	100	97	100	100	100
Sex									
Female	122	100	100	100	97	84	97	98	99
Male	99	100	100	100	88	77	100	98	100
Ethnic ²									
M.A.	151	100	100	100	94	88	98	99	100
Other	70	100	100	100	91	64	99	96	99

¹ DDT-R = total DDT + 1.114 (DDE + TDE).

² M.A. = Spanish surname; other = Non-Spanish surname.

There are no sex differences in residue occurrence, but people with Spanish surnames have higher residues (total DDT, DDE, *p,p'*-DDT, and dieldrin) than people with non-Spanish surnames ($p < 0.001$). Classification by Spanish surname is used as an approximation of Mexican heritage and is certainly only an approximation of ethnic origin.

The mean sample storage intervals were 1.8 months in 1969, 6.7 months in 1970, 11.0 months in 1971, and 6.1 months in 1972. In spite of these rather marked differences there was no correlation between this variable and the residue levels. The mean ages for each year (56 for 1969, 55 for 1970, 54 for 1971, and 52 for 1972) suggest the samples were selected from the same population. There were also no differences in distribution of ethnic group, sex, or type of surgery for any of the study years.

The type of surgery from which our adipose samples were drawn is shown in Table 3. The proportion of lipid in the samples averaged 77.2%. There was no correlation between age and pesticide residues; almost all tissue samples were from adults. Previous reports have shown age correlations in only the youngest groups (3).

TABLE 3.—Classification of elective surgery supplying adipose tissue—Texas, 1969-72

Inguinal hernia	44%
Umbilical hernia	27%
Incisional hernia	13%
Hernia, other	4%
Lipoma	6%
Other types of surgery	6%
	100%

The detection of PCB's is detailed in Table 4; surprisingly, these residues appeared during one year only: 1971. This group of 15 people, compared to people not having PCB's, did not differ significantly in sex, ethnic background, sample storage time, surgical procedure, or

TABLE 4.—Polychlorinated biphenyls in human adipose tissue from elective surgery—Texas, 1969-72

POLYCHLORINATED BIPHENYLS (0 = >0.5 PPM)				
	NO. SAMPLES	% POSI- TIVE	MEAN, PPM	RANGE, PPM
Total	221	7	0.1	0-9.9
Year				
1969	26	0	0	0
1970	68	0	0	0
1971	88	15	1.7	0.6-9.9
1972	39	0	0	0
Sex (1971 only)				
Male	42	24	2.3	0.6-9.9
Female	46	11	0.9	0.6-1.0
Ethnic Background ¹ (1971 only)				
Mex.-Amer.	61	15	0.9	0.6-1.4
Anglo-Amer.	27	22	2.9	0.8-9.9

¹ By surname.

level of pesticide residues; but they were slightly older: 62.5 years vs. 51.5 years ($p < 0.01$).

Graphic analysis of the distribution of the seven residues showed them to be skewed somewhat to the right as has been demonstrated previously (19). The mean is used as the measure of central tendency for comparison to previous work rather than as the rigorous application of statistical principles.

Discussion

Although use of DDT and most other organochlorine pesticides in the Rio Grande Valley was discontinued one year before this study began, the storage level did not decrease among Rio Grande Valley residents. These pesticides have been replaced by organophosphates. These findings confirm the experience of Morgan and Roan (9) but contradict Warnich's findings in Utah (12).

Duggan's food monitoring from 1965 to 1970 shows an apparent decrease in residue levels in food (20, 21), the major source of pesticide intake. This decrease, combined with a decrease in local use on cotton, should result in a detectable decrease in pesticide levels of human adipose, considering Morgan and Roan's (22) demonstration that storage levels are dependent upon continuing intake. The exact changes that may have occurred in pesticide intake via food for our study group are unknown, although it is evident from the data that no dramatic decrease has occurred.

The significant ($p < 0.01$) increase in DDE levels between 1971 and 1972 is a perplexing find. There was no corresponding increase, during that year, in TDE or DDT levels as would be expected if there had been an increase in intake via food. In fact, the National Food Monitoring Program has shown that DDT levels are higher than DDE levels for almost all samples examined (20, 21).

The conversion of DDT to DDE in vivo has been shown to proceed slowly (22) and the stability of DDT levels makes this conversion to DDE unlikely.

A possible explanation for an increase in DDE levels alone could result from the discontinuation of DDT usage in the United States. This would cause an increase in the proportion of DDE, and a subsequent increase in the proportion of DDE found in human adipose. But this theory implies an increased exposure of humans to an environmental source of DDE, which seems unlikely. Further monitoring will be necessary to establish this hypothesis.

The observed differences in DDE, *p,p'*-DDT, total DDT, and dieldrin between Mexican-Americans and Anglo-

Americans probably relate to socioeconomic factors rather than to any genetic differences. This conclusion is based on two studies. Davies (15) has attributed increased DDT residues in blacks primarily to their lower socioeconomic status rather than to their race; it has been observed (23) that in the Rio Grande Valley a disproportionate percentage of the lower socioeconomic group are Mexican-American.

Since it is unlikely that Mexican-Americans consume a larger amount of animal products, which have been shown to contribute to the bulk of dietary pesticide intake (20, 21), the difference in residues could relate to employment, home use of pesticides, or other factors associated with lower socioeconomic status. The lack of information in this area discourages further speculation.

A comparison between the data of this report and the residue levels which have been reported previously reveals that these are the second-highest levels of total DDT (2) and the highest levels of DDE yet reported in a general population. The dieldrin and β BHC levels are also high but the heptachlor epoxide levels are not remarkable (1-15, 21).

National and international comparisons of specimens in a study of this type are subject to substantial hazards. Some specimens are from biopsies and others are from autopsies. Samples received are handled in various ways, including storage in formaldehyde, freezing in plastic, or freezing in glass. There are significant differences in analytical technique; data are reported as means, medians, and geometric means. Some data are reported on a wet-weight basis and some on extractable-lipid basis. Some authors correct for recovery, and the computation of total DDT sometimes fails to consider a difference in molecular weight of DDE and TDE. At times, the work done in these areas is not even mentioned. However, many of the laboratories generating the data cited above are operating under the same contract and quality control program as is this laboratory; comparisons with such laboratories should be valid.

The etiology of the high residues in our study group is probably intense pesticide usage necessitated by a combination of local agricultural needs and a climate favorable to insects. Exactly how exposure occurs is obscure. Reporting residues on a total extractable lipid basis tends to make our results uniformly about 20 to 25% greater than data determined on a tissue-weight basis but the variability of lipid in adipose samples (31 to 93%) makes this a reasonable correction.

The detection of PCB's in only one year is difficult to explain. The study area is mostly rural with very little industrialization and consequently few sources of PCB's. It is possible that these residues represent immigrants

from industrial areas but this has not been demonstrated; as stated earlier, it was not possible to determine a pesticide exposure history of persons in this study. Analysis of all variables identified in the study fails to demonstrate any significant differences between the people with PCB residues and those without, except that the former are slightly older: 62.5 years vs. 51.5 years ($p < 0.001$).

Residue levels are in the range of those reported in the Tissue Monitoring Program (17) and in the Price-Welch study in Michigan (16) but the prevalence is certainly much lower. Without further data it must be assumed that the detection of PCB's in 1971, only, is a random event with no biological significance.

See Appendix for chemical names used in this paper.

Acknowledgments

The study was initiated by J. S. Wiseman, Ph.D. Analytical work was done by E. D. Gomes. Contributions of these and other members of the Texas Community Pesticide Laboratory are greatly appreciated.

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RESIDUES IN FOOD AND FEED

*Surveys of Mercury Levels in Fish and Other Foods*¹

Robert E. Simpson,² William Horwitz,² and Caesar A. Roy³

ABSTRACT

The Food and Drug Administration (FDA) conducted a series of surveys of the mercury content of food in 1970, 1971, and 1972. The surveys included a wide variety of fish samples from selected freshwater regions, commercial fish from wholesale distributors, and swordfish and canned tuna fish; 10 commodities representing a high proportion of total food consumption; and 12 total diet fractions collected in the FDA continuing market basket study to determine pesticide residues in the basic 2-week diet of a 19-year-old male.

For most samples analyzed, atomic absorption was used as the determinative step; the more sensitive neutron activation technique was used to confirm low levels of mercury found by the atomic absorption method.

Swordfish samples showed the highest incidence of mercury and the average mercury content, with levels in excess of 1 ppm in more than 50% of the samples examined. Approximately 4% of the canned tuna fish contained mercury in excess of the FDA guideline of 0.5 ppm for mercury in fish. Some freshwater species contained elevated levels of mercury traceable to known sources of mercury pollution. Of other commercial fish sampled, halibut, bonito, mackerel, cod, and snapper contained some elevated mercury levels, but averaged below the 0.5 ppm guideline.

In the survey of mercury in foods in which 10 food commodities were analyzed, mercury was detected only in shrimp at levels approximating 0.05 ppm. In the total diet fractions only meat, fish, and poultry contained mercury as high as 0.04 ppm. All other fractions contained mercury levels lower than the sensitivity of the method, i.e., 0.002 ppm on a dry-weight basis.

Introduction

The toxic nature of mercury and mercurial compounds has been known for many years. Numerous instances of mercury poisonings resulting from industrial accidents have been reported, but the problem of mercury residues in foods as a result of industrial environmental contamination has been recognized only recently.

In July 1969 the Food and Drug Administration (FDA), alerted by reports of the mercury poisonings in Minamata and Niigata, Japan, resulting from environmental pollution, established an administrative guideline of 0.5 ppm for mercury in fish, shellfish, and other aquatic organisms. This guideline represents the level at which the FDA will charge that the fish is adulterated and must be removed from the market.

The FDA, assisted by study groups of scientists from within and outside the agency, has re-evaluated the guideline on at least three occasions. One study group, in its report on hazards of mercury (1) to the Pesticide Advisory Committee of the Secretary of the U.S. Department of Health, Education, and Welfare (HEW), concluded "... the interim FDA guideline of 0.5 ppm mercury in fish is, for the present, a sound basis for the protection of public health ..."

Mercury was included as one of the toxic contaminants to be surveyed in the FDA compliance program, "Pesticide Residues—Total Diet Studies," as early as 1967 (2). It was also included in that program for the years 1969, 1970, and 1971.

In March 1970 FDA officials learned of a potential mercury pollution problem involving certain Canadian—American border lakes. A compliance program, "Mercury Contamination in Fish" (3) was initiated in April

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1970 to assess the impact of the mercury pollution on sport and commercial fish in freshwater rivers and lakes across the country.

In October of that year Jervis et al., using neutron activation analysis, reported high mercury levels in wheat, flour, and milk products in Canada (4). This report prompted the FDA to initiate a survey of mercury in foods (5) in which certain high-consumption food items were examined for mercury to obtain more information about the exposure of American consumers to mercury in the diet.

The following December, McDuffie (6) reported that samples of tuna examined in his laboratory showed mercury levels that exceeded the 0.5 ppm guideline. On the basis of similar findings by FDA laboratories, a joint FDA—National Cannery Association (NCA) survey of mercury in tuna was initiated to examine samples from all the Nation's domestic and imported canned tuna. The survey was expanded in mid-December 1970 to include swordfish after a preliminary sampling of swordfish stocks showed a substantial number of samples that exceeded the guideline.

In January 1971, a survey of mercury in wholesale fish (7) was instituted to determine the extent of mercury contamination in 19 of the leading commercial fish species.

This paper summarizes results of the FDA survey programs and the various analytical methods used in examining the large numbers of samples.

Sampling and Preparation of Samples

The survey for mercury contamination in fish (3) initiated in the spring of 1970 included samples from the 17 FDA districts whose locations are given in Figure 1. Inspectors from each district investigated various plants engaged in the chlorine-alkali, vinyl-chloride, and other manufacturing processes which used mercury compounds. They identified the freshwater areas used for waste disposal, and collected fish from these areas. Preference was given to bottom-feeders, commercial and sport fish, and other edible fish, including predators. Whenever possible, at least six fish with a total weight of at least 1 lb were collected from each area. Mercury in the samples was determined by the district laboratory in whose territory the sample was collected.

Samples for the survey of mercury in foods (5) were collected by each FDA district. Two samples of the ten commodities, each weighing at least 2 lb, were obtained from local retail markets, preferably representing different manufacturers. If more than one package was required to meet the minimum weight, multiple units of

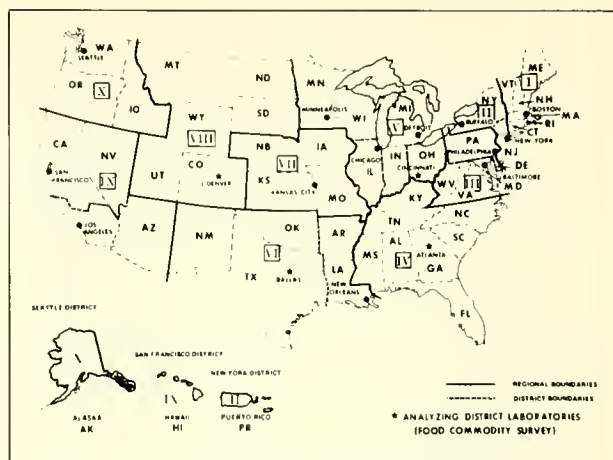


FIGURE 1.—Food and Drug Administration districts within U.S. DHEW regions

the same code were collected. All samples were forwarded as perishables under solid carbon dioxide by the collecting districts to one of the four analyzing districts' laboratories indicated by a star in Figure 1. The analyzing laboratories prepared composites of each commodity collected at a given district; a portion of each composite was forwarded to the FDA headquarters laboratory in Washington, D.C., for mercury determination by neutron activation analysis (NAA).

In the FDA-NCA survey of canned tuna fish and swordfish, samples were collected from warehouse stocks located in each district; samples consisted of 12 cans selected at random from different cases within a given lot.

For the survey of wholesale fish (7), 19 different types of other commercial fish and shellfish were sampled. Each sample consisted of five 1-lb units, each unit from a different fish, making a total of 5 lb for each lot. For shellfish, the square root of the number of containers in the lot was sampled; a minimum of 5 and a maximum of 15 subdivisions were taken. Each subsample consisted of at least 1 lb. The shellfish sampling procedure was also used for packaged fish: fresh, frozen, or processed. Each subsample contributed equally to a single thoroughly mixed slurry for analysis which was representative of the lot.

The Total Diet program (8) of FDA is a continuing market basket study in which 117 food items are collected and composited into 12 commodity groups for analysis. The program is intended to represent the 2-week diet of a 15- to 20-year-old male in each of the four regions of the country (Fig. 1) in which the samples are collected: Northeastern (I and II), Southern (III, IV, and VI), Central (V and VII), and Western (VIII, IX, X). The details of the selection, collection, preparation, and compositing of the samples are described in

references 8 and 9. The homogenized, slurried composites of each commodity group were divided into 100-g portions and kept frozen until analyzed by the Kansas City District laboratory. In addition, portions of each commodity group were forwarded to the FDA headquarters laboratory in Washington for mercury analysis and multi-element screening by neutron activation techniques.

Analytical Methods

Originally the dithizone method of the Association of Official Analytical Chemists (AOAC) (10) and the cold vapor atomic absorption method of the Fisheries Research Board of Canada (3) were used to analyze fish for mercury. When the results of the collaborative study conducted by Munns and Holland (11) became available in late 1970, all subsequent analyses were made by their modification of the basic cold vapor atomic absorption method. The very low levels of mercury in foods other than fish necessitated the use of the more sensitive neutron activation method of evaluation.

DITHIZONE METHOD

The official final action AOAC method (10) consists of a nitric-sulfuric acid digestion under reflux to avoid loss of mercury. The mercury is isolated by dithizone extraction, any interfering copper is removed, and the mercury dithizonate is determined spectrophotometrically. Extreme care is required to achieve complete digestion, or results will be low. The practical limit of detectability is about 0.05 ppm. Recoveries by this method at levels of 0.2 ppm or greater ordinarily are above 90%.

ATOMIC ABSORPTION METHODS

The original Canadian method of analysis involved sulfuric acid dissolution of the sample, cold permanganate oxidation, reduction and volatilization of the mercury, and reading in a vapor-flow cell. The current first action AOAC modification of this method (11) utilizes a nitric-sulfuric-perchloric acid digestion under reflux, reduction and volatilization, and reading in a vapor-flow cell. The practical limit of quantitative results with this method is about 0.02 ppm. Recoveries of mercury added to fish are about 80% at the 0.5 ppm level, with a coefficient of variation of about 15% (11). The average and standard deviation obtained by 17 FDA laboratories for a ground, composited, reference sample of canned tuna, oil packed, was 0.34 ± 0.05 ppm; the coefficient of variation was 15%, about the same as the collaborative study value (11).

RADIOCHEMISTRY METHODS

Older laboratories which at some time have utilized open containers of mercury, such as barometers and leveling bulbs, or any laboratory in which thermometers, calibrating equipment, or bottles of mercury have been

spilled or broken may experience low levels of mercury contamination from hidden sources. The extreme sensitivity of neutron activation analysis therefore required special preparation techniques.

All samples were prepared in a mercury-free clean room. Following irradiation, all sample vials were decontaminated of any possible extraneous radioactive mercury prior to radiochemical processing.

The sulfide precipitation method adapted by Tanner *et al.* (12) from that developed by R. E. Jervis (4) was used to process all samples analyzed in the foods survey program. Sjöstrand's electrolytic method (13) as modified by Heitzman and Simpson (14) was also used to obtain comparative values with other methods on a limited number of selected samples.

The mercury content of the samples was determined by assaying the ^{203}Hg activity using the techniques of gamma ray scintillation spectrometry.

Following a radiochemical separation, the sensitivity of neutron activation analysis for mercury is about 0.002 ppm for dry samples and about 0.001 ppm for freeze-dried samples calculated to the original wet-weight basis. All results were corrected by the individual recovery factor for the specific determination. The average recovery factor was 88%. All results on freeze-dried samples were recalculated to the original wet-weight basis.

Results and Discussion

COMPARISON OF METHODS

A number of samples which had been analyzed by several other laboratories were available for comparative analyses. Although some results of this study have been reported elsewhere (12, 14), for the sake of completeness they have been included in Table 1 with additional data. The concurrence of results between neutron activation analyses and atomic absorption methods, which measure total mercury, and the gas-liquid chromatographic (GLC) analyses, which measure only methyl mercury, indicates (a) that most of the mercury in the shrimp and two pike samples is present as methyl mercury, and (b) that mercury is not lost in freeze drying.

The electrodeposition method yields mercury of high purity deposited on gold, and the results are in good agreement with those by the other methods. This technique, however, is not practical for processing the large number of samples handled in these surveys.

The results in Table 1 show remarkably good agreement by the various laboratories utilizing their individual

techniques. In all cases, special care undoubtedly was taken to insure the reliability of the reported values.

FISH SURVEY

A survey was initiated by the U.S. Department of the Interior—Federal Water Quality Administration (FWQA: a predecessor of the present U.S. Environmental Protection Agency—Air and Water Programs). J. R. Harlan, Conservation Consultant to FWQA, sent an inquiry to all State directors of fish, game, and conservation, soliciting information on the status of mercury pollution of natural resources under their jurisdictions (15). Results reported by the States are given in Table 2. Of the 45 States who responded to the inquiry, 13 reported levels of mercury in fish in excess of 0.5 ppm; at least six States reported levels higher than 4 ppm in some samples. Closures of fishing areas and/or warnings against eating fish from these polluted areas were issued by 16 of the States covered in the survey.

In Lake Erie (Table 3) over 20% of the fish sampled by FDA in 1970 contained mercury in excess of the 0.5 ppm guideline; over 50% of the fish from Lake St. Clair in nearly Ontario Province, Canada, were above this level. Of these, 20% showed more than 1 ppm mercury. Fish from other regions, except for Lake Ontario, had residues well below this level. For comparison, the mercury contents of fish from two Far Western areas are also listed in Table 3. More than 60% of all samples analyzed contained less than 0.1 ppm mercury. The official dithizone method of the AOAC (10) was used to analyze most samples collected in this survey. After adoption of the Munns and Holland method (11), about 60% of the Lake Erie samples were analyzed by atomic absorption. Results for the fish from Lake Erie and Lake St. Clair confirm the earlier Canadian findings: walleye, sheepshead, catfish, white bass, smallmouth bass, some perch, and carp contained the highest levels of mercury.

Other data from the FDA survey of mercury contamination in fish are given in Table 4. Of 29 freshwater areas listed, only 13 had fish containing mercury in excess of the 0.5 ppm guideline; of these, only 9 areas had fish containing more than 1 ppm. Samples from all other areas contained less than 0.5 ppm mercury.

The 1971-72 results for 13 different types of fish are shown in Figure 2. Of all the samples collected from various parts of the country, 40% (16 of 40 samples) contained levels of mercury in excess of 0.5 ppm. As illustrated in Figure 3, similar results were observed for imported freshwater fish. In this case about 45% (7 of 16 samples) contained more than 0.5 ppm.

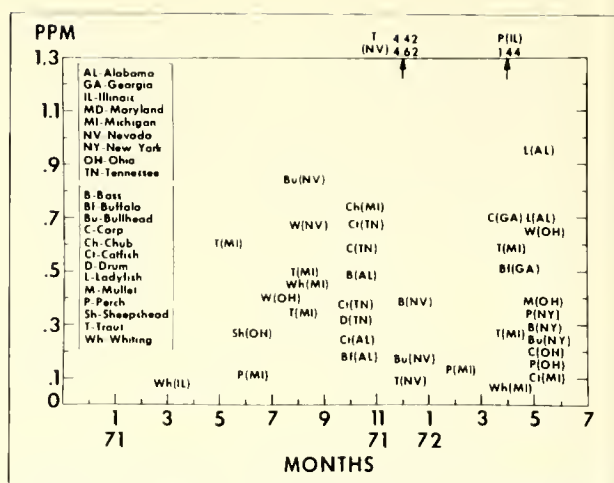


FIGURE 2.—Mercury levels in domestic freshwater fish, 1970-72

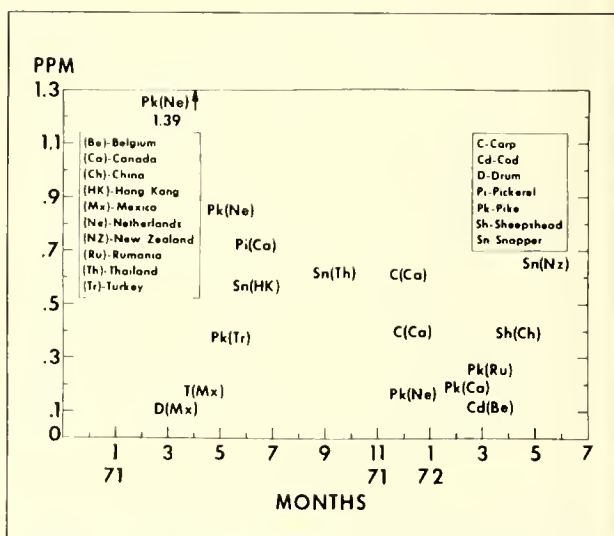


FIGURE 3.—Mercury in imported freshwater fish, 1970-72

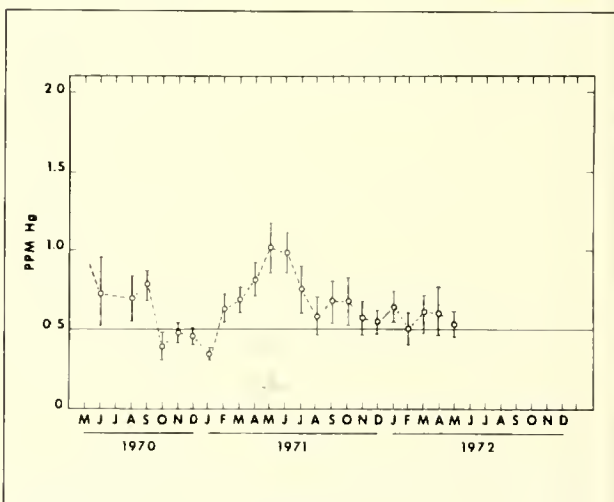


FIGURE 4.—Mercury in fish collected from Pickwick Reservoir, Tennessee Valley Authority

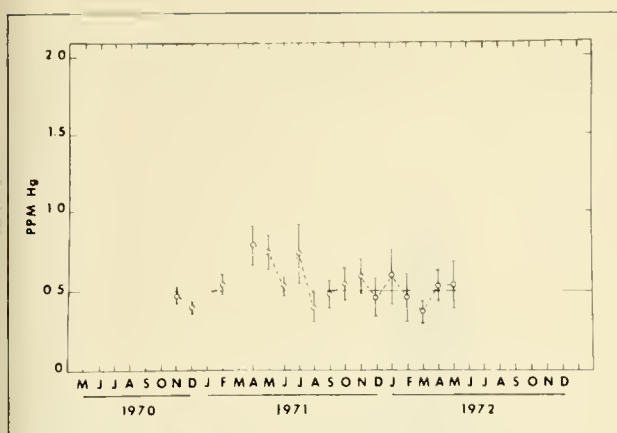


FIGURE 5.—Mercury in fish collected from Kentucky Reservoir, Tennessee Valley Authority

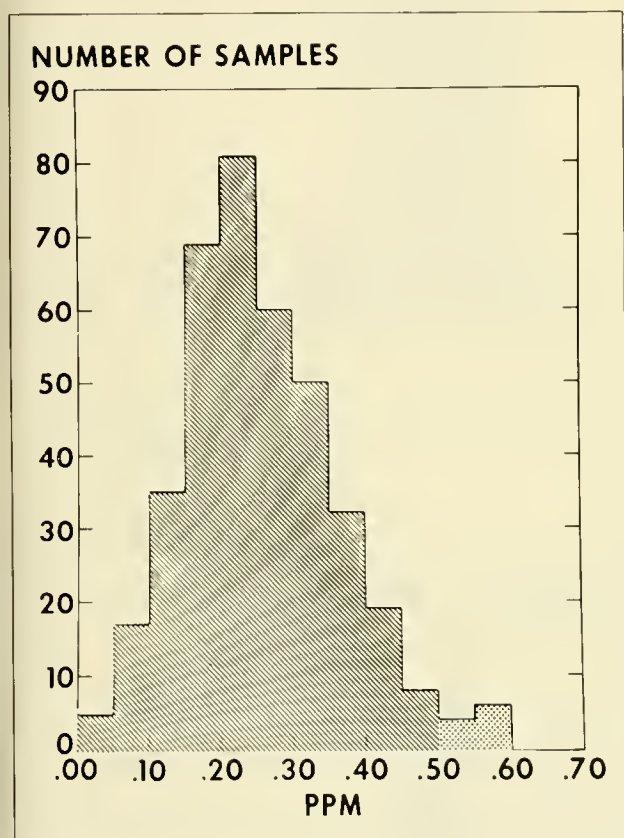


FIGURE 6.—Distribution of mercury in albacore (NCA data)

Since 1967 the Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior, has conducted a monitoring program for pesticides in fish collected from various lakes and streams throughout the United States. Results from an extensive survey for mercury in freshwater fish, recently reported by Henderson, Inglis, and Johnson (16), indicated that in 1969 values ranged from <0.05 to 1.25 ppm and in 1970 from <0.05 to 1.80 ppm total mercury in the edible portions. Of 100

monitoring stations, average values for total mercury exceeded the FDA guideline of 0.5 ppm at three stations in 1969 and at six stations in 1970.

Along with other collaborating organizations monitoring mercury pollution in freshwater areas of the United States, the Water Quality Control Branch (WQCB) of the Tennessee Valley Authority (TVA) has been co-operating with the Food and Drug Administration and the U.S. Environmental Protection Agency (EPA). In a recent report covering their survey of mercury in fish from TVA reservoirs (17), WQCB found significantly high levels of mercury in fish obtained from both the Pickwick, Tennessee, and the Kentucky reservoirs (Fig. 4, 5). After eliminating the discharge of mercury into these waters, TVA researchers have observed a downward trend in mercury concentrations among reservoir fish, though the level remains above 0.5 ppm. As overlaying mercury-free sediments continue to collect in these waters, researchers expect this downward trend to continue.

CANNED TUNA FISH

Analyses of over 3,000 samples of canned tuna (Table 5) show an average overall mercury content of about 0.25 ppm, with less than 4% over the 0.5 ppm guideline. The coefficient of correlation between weight of fish and mercury level is given for each type of fish and for all types combined for the National Canners Association (NCA) data. Weights were not available for the imported samples, and such correlations could not be examined. This correlation is highly significant in domestic samples for albacore ($p < 0.001$), yellow fin ($p < 0.001$), the "other" category ($p < 0.01$), and all types combined ($p < 0.0001$), indicating that the mercury concentration increases with an increase in fish size. The correlation for skipjack was not significant, perhaps because the largest weight for the skipjack was 25 lb. and the increased opportunity of the larger fish to concentrate the mercury may be more manifest in fish larger than 25 lb. Figures 6 and 7 show that the mercury concentration in albacore and skipjack tuna approaches a normal distribution; the larger albacore has the greater number of samples with elevated levels of mercury. Only in imported tuna (Fig. 8) is the distribution skewed above 0.5 ppm. More recent results illustrated in Figure 9, however, suggest a downward trend for both domestic packs and imported tuna. This probably indicates closer scrutiny by the suppliers, rather than any real reduction of mercury concentration in these fish species.

HALIBUT

During the 1971-72 mercury survey, FDA analyzed more than 500 samples of domestic and imported halibut (Table 6). The overall average concentration of mercury was about 0.25 ppm, and about 13% of the samples were above the 0.5 ppm guideline.

SWORDFISH

Results for swordfish are quite different from those for halibut. Of 853 samples analyzed in 1971 (Table 7), over 95% exceeded the 0.5 ppm guideline and over 50% of the samples exceeded 1 ppm mercury. As Figure 10 shows, however, the concentration approaches a normal distribution, as in tuna, with the greatest number in the range 0.75-0.99 ppm. As a result of these findings, FDA recommended that the public not eat swordfish and that this commodity be recalled from the domestic market (1). Commercial swordfish usually average about 150 lb each; hence these findings are not surprising in view of the correlations with weight in tuna, as mentioned earlier.

COMMERCIAL FISH AND SEAFOOD

Among the more than 1,300 samples of 19 different types of other commercial fish and seafood collected in 1971, only six types included one or more fish with a mercury content above the 0.5 ppm guideline (Table 8). Of those species containing a significant number of samples, bonita and snapper averaged more than 0.2 ppm mercury. Although a few crab, cod, flounder, lobster, and herring reached this level, the high-level samples constituted less than 10% within each type.

SURVEY OF MERCURY IN FOOD (5)

Of all food examined for mercury content, measurable quantities were found only in shrimp when examined by the neutron activation technique (12); it had a high value of 0.043 ppm and a median value of 0.014 ppm (Table 9). Nonfat dry milk was next highest with a high of 0.027 ppm and a median of 0.010 ppm. The mercury level in nonfat dry milk, when reconstituted, is of the same order as that in fluid whole milk: approximately 0.002 ppm. All commodities except shrimp were also analyzed by the dithizone and the atomic absorption methods, which were not sensitive enough to measure with absolute precision such low levels of mercury. Table 9 gives the median and range of values in the 10 commodities found by the neutron activation method only.

TOTAL DIET SURVEYS

Results from the most recent total diet survey by the current neutron activation analysis method (12) are presented in Table 10 with results reported previously (2). All commodities except meat, fish, and poultry (Group II) contained less than 0.014 ppm mercury. The meat, fish, and poultry group contained mercury ranging from 0.004 to 0.041 ppm, depending upon the mercury level in the fish component. The amount of mercury currently found in red meat muscle tissue is about 0.010 ppm (1972, A. J. Spaulding, United States Department of Agriculture, *personal communication.*), comparable to the levels generally found in Fraction II.

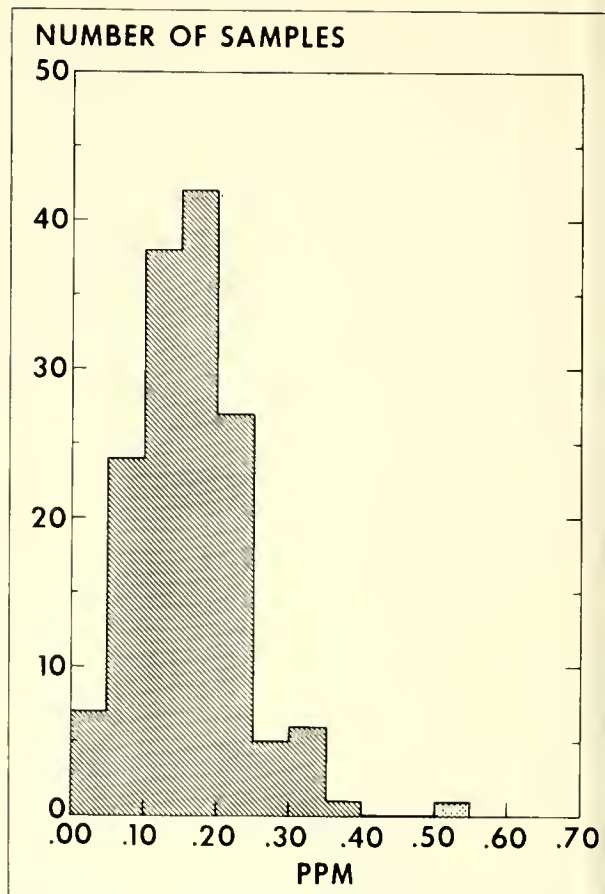


FIGURE 7.—Distribution of mercury in skipjack tuna (NCA)

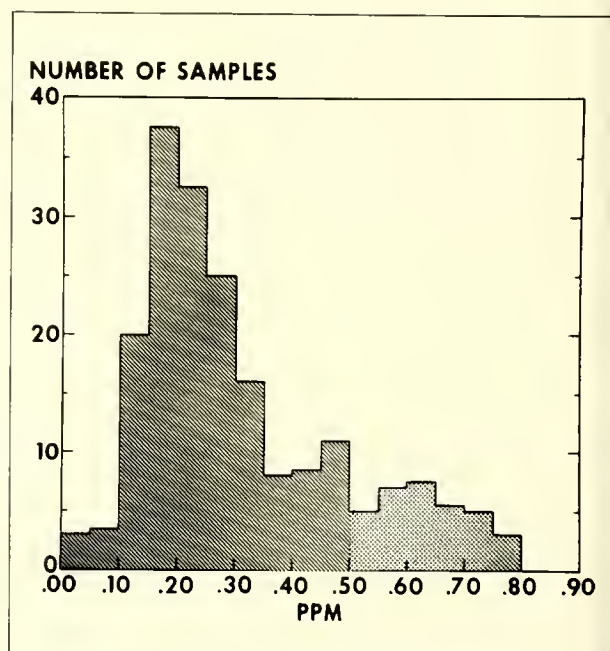


FIGURE 8.—Distribution of mercury in imported tuna

Of all commodities analyzed for mercury in the surveys reported in this paper, fish appears to present the only potential hazard to man. However, levels of mercury that exceed the 0.5 ppm guideline for fish are found only in the larger species (large tuna, halibut, and swordfish) of the commercially important varieties and in fish obtained from waters of known mercury pollution. In all other commodities, mercury either is not detectable or is present at levels approximating two orders of magnitude lower than the guideline for fish.

Acknowledgments

1. Data from the fish and foods survey were collated by the Office of Compliance, U.S. DHEW-FDA, with special cooperation of Mary J. Dolan. The FDA District inspectors and laboratory personnel (Fig. 1) who collected and analyzed the samples were responsible for the success of these surveys.
2. James Winbush, Janet Springer, and other members of the mathematical staff have assisted greatly in statistically evaluating data. Fish data were processed by Daryl C. Fleming of the Systems Analysis Branch, Office of Executive Director of Regional Operations.
3. The neutron activation analysis results of the foods survey were acquired through the efforts of James T. Tanner and colleagues (12) in the FDA facilities at the National Bureau of Standards reactor in Gaithersburg, Maryland. These facilities are made available to FDA through an interagency agreement among several agencies: DHEW-FDA and Department of Commerce NBS.
4. We appreciate the cooperation of other organizations mentioned in the text, who permitted their data to complete results of the study.

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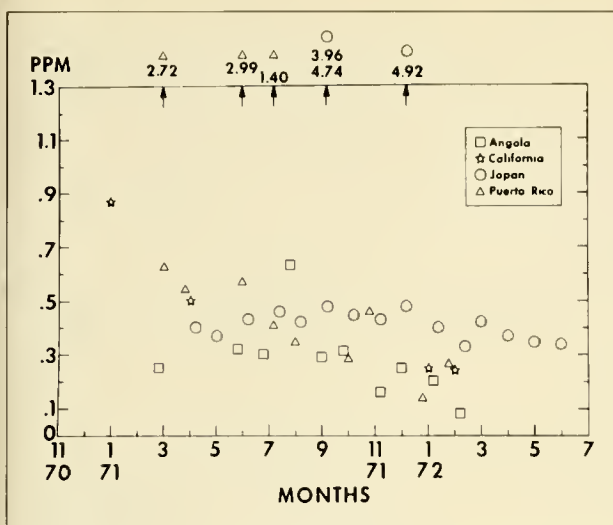


FIGURE 9.—Mercury in both domestic and imported tuna combined

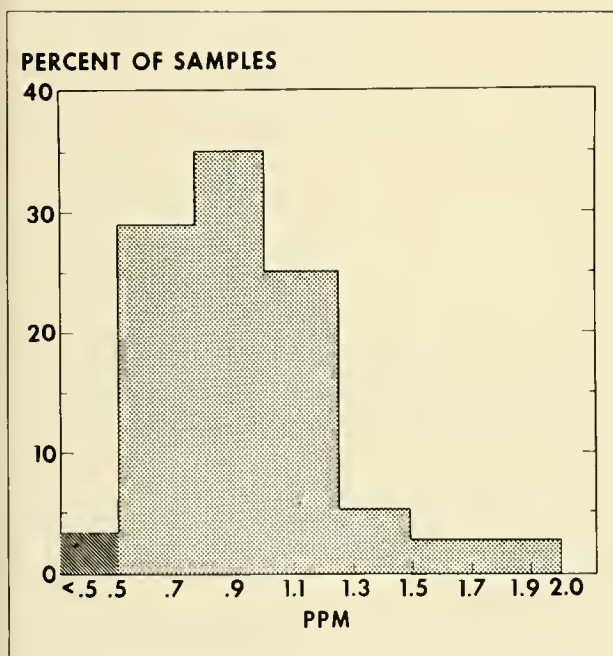


FIGURE 10.—Distribution of mercury in swordfish

The earlier results are higher than current data by one order of magnitude (2). This was probably due to the presence of low-level mercury contamination introduced in earlier sample preparation, the significance of which has been recognized only recently (12). This same factor also may have been responsible for the high results reported earlier in the Jervis study (4).

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TABLE 1.—Comparative results (ppm) of mercury tests, using different analytical methods

COMMODITY	RADIOCHEMISTRY OF NEUTRON ACTIVATED SAMPLES FOR TOTAL MERCURY ¹			OTHER METHODS	
	SULFIDE PRECIPITATION	ELECTROLYTIC DEPOSITION	PYROLYSIS ²	GAS CHROMATOGRAPHY (METHYL MERCURY)	ATOMIC ABSORPTION (TOTAL MERCURY)
FDA shrimp	0.033 ± 0.003 0.029 ± 0.003 0.026 ± 0.003 0.043 ± 0.004			^a 0.03 0.03 0.04 0.06	⁴ 0.03 0.02 0.00 0.01
Swedish pike ⁶ F-410-28	2.16 ± 0.22	2.24 ± 0.22 ^a 1.94	2.20 ± 0.15	^{3a} 2.19 ± 0.10 ^{3b} 2.22 ± 0.03 ^a 1.88	
Swedish pike F-410-30	1.29 ± 0.13	1.17 ± 0.11 ^a 1.10	1.18 ± 0.15	^{3a} 1.18 ± 0.02 ^{3b} 1.10 ± 0.03 ^a 1.10	
Orchard leaves ⁷	0.148 ± 0.01	0.162 ± 0.02 0.154 ± 0.02 0.169 ± 0.02	0.155 ± 0.015		
CHPB flour ⁸	0.011 ± 0.01 ^a 0.014 ± 0.004	0.012 ± 0.002 0.015 ± 0.002 0.008 ± 0.002	0.011 ± 0.003		⁸ 0.013
FDA flour ¹⁰	0.002 ± 0.002 ¹¹ 0.004				
IAEA flour ¹²	4.6 ± 0.5 4.9 ± 0.3		4.87 ± 0.5		
Bowman kale ¹³	0.25 ± 0.03 ¹³ 0.23				

¹ All FDA neutron activation analysis data represent single determinations; therefore, ± deviations represent counting error and radiochemical uncertainties arbitrarily set at ± 10%.

² Rook, H. L., T. E. Gills, and P. D. Lafleur. 1972. Anal. Chem. 44: 1114.

³ L. R. Kamps. 1971. FDA Headquarters. Private communication. (a) Samples analyzed on a wet basis, average of 3 replicates; (b) samples analyzed after freeze drying, average of duplicates.

⁴ FDA District results. 1971. Office of Compliance Report.

⁶ Pike samples courtesy of National Institute of Public Health, Stockholm, Sweden.

¹⁰ Westoo, G. 1971. Stockholm, Sweden. Private communication.

⁷ NBS Standard Reference #1571. 1971. "Orchard Leaves."

⁸ Flour sample C-32572 courtesy of H. M. Cunningham. 1971. Canadian Health Protection Branch.

⁹ Somers, E. 1971. Proceedings Mercury Conference, Ottawa, Canada.

¹⁰ FDA flour survey sample. 1971.

¹¹ Lyon, W. 1971. Oak Ridge National Laboratory. Private communication.

¹² IAEA Standard Flour 66/10. 1969.

¹³ Nadkarni, R. A., and W. D. Ehmann. 1969. J. Radioanal. Chem. 3:175-185.

TABLE 2.—Mercury in freshwater fish¹

STATE	AREAS	FINDINGS	ACTIONS
Alabama	Pickwick Reservoir, tributaries of Tennessee River, Tombigbee River	Trace to 0.5 ppm	Closed to commercial fishing
Arizona		No findings reported	
California	Sacramento-San Joaquin Delta Clear Lake	Up to 1.27 ppm	No closures
Colorado	Eastern irrigation reservoir	0.04 to 0.08 ppm	No closures
Connecticut		No findings reported	
Delaware	Delaware River, Delaware Bay	<0.5 ppm	No closures
Florida	Pulp mill areas	No Hg found ²	—
Georgia	Fresh and estuary waters	Not reported	Savannah River and Brunswick Estuary closed
Hawaii		No findings reported	
Idaho		No findings reported	

TABLE 2.—Mercury in freshwater fish¹—Continued

STATE	AREAS	FINDINGS	ACTIONS
Illinois	Mississippi River, St. Clair Co.	Three samplings each: carp, 0.09-15 ppm catfish, 0.44 ppm white bass, 0.20 ppm perch, 0.15 ppm shad, 0.08 ppm	No closures
Indiana	Waters of Lake Michigan	Whole fish, 0.18 ppm ²	No closures
Iowa	Random sampling	0-0.002 ppm ²	No closures
Kansas	Kaw Rivers	Hg found ²	No closures
Kentucky	Tennessee River below Kentucky Dam	0.6 to 1.14 ppm	Stopped commercial fishing in Tennessee River area of Kentucky
Louisiana	Calcasieu Lake	Not reported	Warning against eating lake fish
Massachusetts	Launton River area	Fish, 0.17-1.21 ppm; shellfish, mussels, clam, 0.3-4 ppm	No closures
Michigan	St. Clair River Wyandotte, Detroit River	0-7 ppm ²	Commercial closure of St. Clair River, Lake St. Clair, Lake Erie
Minnesota	Statewide	<0.5 ppm except Red River of the North	No closures
Mississippi	Unspecified	0.05-1.14 ppm (in Alabama)	No closures
Missouri	No known Hg pollution sources ²		
Montana	Above and below pulp mill	Trace ²	No closures
Nebraska		No findings reported	
Nevada		No findings reported	
New Hampshire	Connecticut River Merrimack River	Of fish analyzed, 1/3 >0.5 ppm; 1/4 >0.5 ppm; highest, 1.3 ppm	No closures
New Jersey	Arthur Kill River	0.03-0.21 ppm	No closures
New Mexico	Navajo Lake	All varieties, 0-0.16 ppm; brown trout, 1.14-1.16 ppm; bullheads, 0.68 ppm; catfish & trout, 0.34 ppm; bluegills, 0.4 ppm	
	Avalon Reservoir Elephant Butte Reservoir Animas River	0.05-0.22 ppm 0.14-0.7 ppm Catfish & trout, 0.13 ppm	Warning against eating fish
New York	Statewide	Range 0.01-8.2 ppm	Warning against eating fish; Lake Ontario; Lake Champlain; Lake Erie; St. Lawrence, Oswego & Niagara Rivers
North Carolina	Cape Fear River	Hg contamination found ²	No closures but monitoring of inland areas initiated
Ohio	Kiser Lake Buckeye Lake Maumee River Lake Erie	Walleye, 0.55 ppm White bass, 0.59 ppm	
	Western Basin	Range 0.04-1.51 ppm	Lake Erie closed to commercial fishing; warning to others
	Vermilion area Cleveland area	Coho, 0.24 ppm; walleye, 0.62-1.4; white bass, 0.8 ppm; perch, 0.48-0.78 ppm; carp, 0.67 ppm; mullet, 0.47 ppm; drum, 0.29-0.65 ppm; catfish, 0.07-4.6 ppm	
		Perch, 0.22 ppm; carp, 0.22 ppm	
		Perch, 0.55 ppm; mullet, 0.5 ppm	
Oklahoma		No findings reported	
Oregon	Unspecified	No Hg found	
Pennsylvania	Lake Erie only Hg source	0.5-3 ppm	Warning against Lake Erie fish
Rhode Island	Unspecified	No Hg found	
South Carolina		No findings reported	Savannah River closed Augusta to Coast
South Dakota	Oake Reservoir, Cheyenne River Impoundment to Missouri River	0.03-0.35 ppm	No closures
Tennessee	Pickwick Lake	No data cited	Closed to commercial fishing
Texas	Houston Ship Channel Lavaca Bay	Waters, 19 ppm (?) oysters >5 ppm	Lavaca Bay closed
Utah	Limited survey	0.03-0.06 ppm	No closures
Vermont	Lake Champlain Other lakes and ponds	1/2 of fish samples >0.5 ppm 1/4 >0.5 ppm	Closed to commercial fishing
Virginia		No findings reported	Warning against fish from N. Fork Holston River
Washington	Unspecified	Unspecified	No waters closed in any areas
West Virginia	Ohio and Monongahela Rivers	No data	Closure of these reservoirs
Wisconsin	Wisconsin River of known Hg use	High as 4.62 ppm	Restraint warning
Wyoming	Ocean Lake Reservoir; Alcova Reservoir	0.05-0.116 ppm	No closures

¹ Survey initiated by USDI—Federal Water Quality Administration (FWQA), 1970.² Reported by R. H. Stroud. 1971. Marine Sport Fisheries Research. Sport Fishing Inst. Bull. No. 221 (15).

TABLE 3.—Mercury in Great Lakes fish, 1970

REGION	TYPE	No. OF SAMPLES	MEAN $\pm$ STANDARD DEVIATION (PPM)	PERCENT IN PPM RANGES OF:				
				<0.1	0.1-0.50	0.51-1	>1	>2
Lake Erie	Walleye	26	0.58 $\pm$ 0.26	4	27	61	8	2
	Perch	53	0.24 $\pm$ 0.14	8	85	7	0	—
	White bass	37	0.49 $\pm$ 0.31	6	38	48	8	0
	Sheepshead	29	0.28 $\pm$ 0.21	15	65	20	0	—
	Carp	19	0.28 $\pm$ 0.29	17	75	4	4	0
	Mullet	11	0.21 $\pm$ 0.16	27	73	0	0	—
	Sucker	14	0.21 $\pm$ 0.13	21	79	0	0	—
	Catfish	20	0.23 $\pm$ 0.20	30	55	15	0	—
	Smallmouth bass	13	0.51 $\pm$ 0.19	0	69	31	0	—
	All others	55	0.19 $\pm$ 0.23	44	49	7	0	—
Lake St. Clair (Ontario Province, Canada)	Perch	18	0.88 $\pm$ 0.75	0	40	30	30	2
	All others	32	0.48 $\pm$ 0.32	3	56	34	7	0
Lake Michigan	All types	40	0.11 $\pm$ 0.11	62	38	0	0	—
Lake Ontario	All types	74	0.30 $\pm$ 0.30	12	74	10	4	0
Lake Huron	All types	23	0.19 $\pm$ 0.11	22	78	0	0	—
Lake Superior	All types	10	0.13 $\pm$ 0.11	20	80	0	0	—
Columbia River ¹	All types	27	0.05 $\pm$ 0.07	63	37	0	0	—
Puget Sound ¹	All types	11	0.03 $\pm$ 0.07	82	18	0	0	—

¹ These samples were collected from assumed uncontaminated Western areas for comparison with Great Lakes fish.

TABLE 4.—Mercury in fish from various freshwater areas, 1970

REGION	TYPE OF FISH	NUMBER OF SAMPLES ¹	MEAN $\pm$ STANDARD DEVIATION (PPM)	PERCENT IN PPM RANGES OF:				
				<0.1	0.1-0.50	0.51-1	>1	>2
Savannah River, Augusta, Ga.	All types	12	0.56 $\pm$ 0.26	11	32	57	0	—
Beaverhead River, Mont.	All types	10	0.57 $\pm$ 0.34	0	60	30	10	0
Belle Pourche, R.I.	All types	12	0.22 $\pm$ 0.22	42	50	8	0	—
Calcasieu Lake, La.	All types	16	0.39 $\pm$ 0.41	31	31	31	7	0
Cape Fear River, N.C.	Catfish	9	1.14 $\pm$ 0.59	0	12	33	55	0
Cherokee, Tenn., and Boone, Ky., Reservoir	All types	13	0.15 $\pm$ 0.14	46	54	0	0	—
Chocooloco Creek	All types	8	0.39 $\pm$ 0.16	0	75	25	0	—
Lake Charles, La.	All types	23	0.31 $\pm$ 0.35	22	65	9	4	0
Mississippi River	All types	54	0.22 $\pm$ 0.28	37	48	9	6	0
Muscle Shoals, Ala.	All types	6	0.97 $\pm$ 0.75	0	34	33	33	1
Niagara River	All types	8	0.15 $\pm$ 0.07	1	99	0	0	—
Oake Reservoir, Dakotas	All types	12	0.29 $\pm$ 0.24	0	84	16	0	—
Riegelwood, N.C.	Catfish	14	0.84 $\pm$ 0.27	0	8	71	21	0
	Others	12	1.03 $\pm$ 0.56	0	8	42	50	0
Red River, Texas	All types	10	0.17 $\pm$ 0.09	20	80	0	0	—
Sheffield, Ala.	All types	8	1.00 $\pm$ 0.89	0	38	12	50	2
St. Lawrence River	All types	9	1.16 $\pm$ 0.50	0	12	44	44	1
Tennessee River	All types	11	0.20 $\pm$ 0.12	18	82	0	0	—
Wheeler Lake, Ala.	All types	7	0.29 $\pm$ 0.09	0	100	0	0	—
Whitlock Bay	All types	7	0.13 $\pm$ 0.05	27	73	0	0	—
Yellowstone and Tonque Reservoirs, Mont.	All types	10	0.16 $\pm$ 0.08	40	60	0	0	—
Nine other areas ²	All types	29	0.06 $\pm$ 0.03	86	18	0	0	—

¹ The atomic absorption method was used to analyze samples from all but three sources: Calcasieu Lake, Lake Charles, and the Mississippi River.

² Each body of water had 3-5 samples.

TABLE 5.—Mercury in tuna fish

	SKIPJACK	ALBACORE	YELLOWFIN	OTHER
IMPORTED SAMPLES PROCESSED BY FDA				
Number in sample	936	1437	135	200
Mean mercury level (ppm)	0.20	0.31	0.17	0.31
Standard deviation	0.085	0.11	0.097	0.18
Upper 99% confidence limit on mean	0.21	0.314	0.19	0.34
Upper 99% tolerance limit on 99% of population	0.41	0.59	0.44	0.80
Upper 99% tolerance limit on 95% of population	0.35	0.51	0.36	0.67
DOMESTIC SAMPLES PROCESSED BY NATIONAL CANNERS ASSOCIATION				
Number in sample	149	383	288	70
Mean mercury level (ppm)	0.16	0.25	0.21	0.20
Standard deviation	0.078	0.10	0.12	0.12
Upper 99% confidence limit on mean	0.17	0.26	0.23	0.24
Upper 99% tolerance limit on 99% of population	0.37	0.52	0.51	0.55
Upper 99% tolerance limit on 95% of population	0.31	0.44	0.42	0.46
Mean weight ¹	8.18	31.17	50.60	24.86
Correlation coefficient ¹	0.041	² 0.33	² 0.33	³ 0.44, ² 0.67

¹ Based on fewer samples than given above.² Significant at $P < 0.001$.³ Significant at $P < 0.01$.TABLE 6.—Mercury concentration in halibut¹

STATE AND COUNTY, OR COUNTRY	COLLECTION		NO. OF SAMPLES	MERCURY, PPM				NO. OF SAMPLES >0.5 PPM	PERCENT OF SAMPLES >0.5 PPM
	MONTH & YEAR			HIGH	LOW	AVERAGE	$\pm \sigma$		
Alaska, Prince of Wales Co.	8, 10, & 11	71	60	0.92	0.12	0.39	0.19	13	20
California, Alameda Co.	2	71	3	0.16	0.04	0.10			
Los Angeles Co.	2 & 9	71	6	0.37	0.07	0.20			
Illinois, Cook Co.	2 & 3	71	8	0.71	0.05	0.19	0.25	1	
Massachusetts, Suffolk Co.	2	71	4	1.40	0.08	0.41		1	
Oregon, Clatsop Co.	9	71	13	0.41	0.18	0.28	0.08		
Pennsylvania, Phila.	2	71	17	0.29	0.03	0.14	0.07		
Washington, King Co.	7	71	9	0.31	0.07	0.13	0.08		
	8	71	78	0.76	0.08	0.29	0.13	3	4
	9	71	65	1.52	0.13	0.45	0.27	20	30
	10	71	64	1.50	0.07	0.40	0.34	17	27
	11	71	5	0.28	0.07	0.21	0.09		
	5	72	5	0.73	0.60	0.70	0.06	5	100
Washington, Snohomish Co.	10	71	12	0.49	0.11	0.18	0.10		
	11	71	6	0.29	0.18	0.24	0.05		
Washington, Whatcom Co. ²	8	71	23	0.12	0.06	0.12	0.06		
	10	71	27	0.59	0.07	0.23	0.13	3	11
Canada ³	7	71	5	0.39	0.05	0.24	0.15		
	8	71	6	0.13	0.05	0.11	0.06		
	9	71	17	0.46	0.06	0.16	0.10		
	10	71	22	0.26	0.02	0.17	0.07		
	11	71	15	0.74	0.10	0.29	0.21	3	23
	12	71	12	1.10	0.04	0.30	0.28	2	17
	1	72	11	0.49	0.16	0.37	0.11		
	2	72	11	0.49	0.10	0.22	0.14		
	3	72	3	0.30	0.17	0.22	0.07		
	5	72	6	0.49	0.18	0.28	0.14		
	6	72	4	0.25	0.03	0.15	0.09		
Japan	10	71	3	0.31	0.08	0.22	0.12		
	11	71	3	0.42	0.24	0.30	0.1		
	3	72	6	0.32	0.03	0.21	0.09		
	5	72	7	0.25	0.12	0.16	0.05		
	6	72	4	0.12	0.10				

¹ Overall total = 543 samples with an overall mercury concentration = 0.25 ppm with 68 (13%) in excess of 0.5 ppm.² Total sample for Washington State = 294; total >0.5 ppm = 48 (16%).³ Total Canadian sample = 122; total >0.5 ppm = 5 (4%).TABLE 7.—Distribution of mercury levels in swordfish above 0.5-ppm guideline¹

RANGE OF MERCURY LEVELS, PPM	PERCENT OF SAMPLES AT THIS LEVEL
<0.50	—
>0.50-0.74	13.7
0.75-0.99	33.2
1.00-1.24	26.5
1.25-1.49	15.3
1.50-1.99	8.5
2.00-2.99	2.4
3.00->3.00	0.3
	99.9

¹ Of all swordfish sampled, 95% exceeded 0.5 ppm mercury.

TABLE 8.—Mercury in wholesale fish

FISH TYPE	MEAN MERCURY LEVEL, PPM	TOTAL SUB- SAMPLES	NO. OF LOTS	STD. DEV. WITHIN LOTS	STD. DEV. BETWEEN LOTS (COMPONENT OF VARIANCE)	UPPER 99% CONFIDENCE LIMIT ON MEAN	UPPER 95% TOLERANCE LIMIT ON 99% OF LOTS	UPPER 99% TOLERANCE LIMIT ON 99% OF LOTS
Bonita	0.30	41	34	0.04	0.19	0.38	0.87	0.94
Clams	0.03	158	81	0.03	0.03	0.04	0.13	0.13
Cod	0.09	170	80	0.04	0.07	0.11	0.31	0.32
Crab	0.11	236	85	0.09	0.09	0.14	0.39	0.41
Flounder	0.08	156	76	0.03	0.05	0.09	0.23	0.24
Halibut	0.14	151	82	0.03	0.07	0.16	0.38	0.40
Haddock	0.04	179	77	0.01	0.03	0.05	0.13	0.13
Herring	0.07	107	77	0.01	0.05	0.08	0.20	0.21
Lobster	0.07	185	83	0.02	0.06	0.09	0.23	0.24
Mackerel	0.12	140	91	0.11	0.20	0.17	0.70	0.74
Oysters	0.03	203	86	0.02	0.04	0.04	0.15	0.18
Perch	0.06	209	83	0.02	0.06	0.07	0.22	0.23
Salmon	0.04	99	78	0.03	0.02	0.05	0.13	0.14
Sardines	0.02	104	76	0.01	0.02	0.03	0.08	0.08
Scallops	0.02	171	76	0.01	0.02	0.03	0.07	0.08
Snapper	0.31	186	86	0.10	0.15	0.35	0.75	0.78
Trout	0.09	151	83	0.03	0.10	0.12	0.37	0.39
Whitefish	0.05	65	48	0.03	0.03	0.07	0.18	0.19
Whiting	0.06	133	61	0.06	0.04	0.08	0.22	0.23

TABLE 9.—Neutron activation analysis for mercury in foods (12)

COMMODITY	NO. OF SAMPLES	MERCURY RESIDUES IN PPM ²	
		MEDIAN	RANGE
(1) Flour	28	<0.003	<0.003-0.006
(2) Nonfat dry milk	3	0.010	<0.004-0.027
(3) Sugar	22	<0.003	<0.003-0.010
(4) Potatoes	33	0.003	0.001-0.015
(5) Raw ground beef	23	0.003	<0.002-0.007
(6) Chicken breast (raw and boned)	24	0.003	<0.001-0.007
(7) Shrimp	32	0.014	0.005-0.043
(8) Beef liver	22	0.003	<0.002-0.008
(9) Eggs	33	<0.002	<0.002-0.005
(10) Fluid whole milk	32	<0.001	<0.001-0.009

¹ With the exception of (1), (2), and (3), all commodities were freeze dried, but result were calculated on a wet-weight basis using the moisture content for each sample.

² Upper limits represent twice the standard deviation of the background count.

TABLE 10.—Neutron activation analysis for mercury in total diets

COMMODITY GROUPS ²	MERCURY CONCENTRATION (PPM) ¹				
	SOUTHERN REGION ³ (BALTIMORE)	NORTHEAST REGION (BOSTON)	CENTRAL REGION		WESTERN REGION (LOS ANGELES)
			(KANSAS CITY)	(MINNEAPOLIS)	
I Dairy Products	<0.001	<0.001	<0.001	0.003	0.002
II Meat, Fish, and Poultry	0.030	0.013 0.026 0.041	0.006 0.004 0.013	0.009	0.027
III Grain and Cereal Products	0.003	0.012 0.004	0.005	<0.002	<0.002
IV Potatoes	0.003	0.002 0.013	<0.002	0.003	<0.001
V Leafy Vegetables	0.001 0.009	0.003 0.006	0.004 0.002	<0.001	<0.001
VI Legume Vegetables	<0.002	<0.001	<0.002	<0.001	<0.001
VII Root Vegetables	0.002	<0.001	<0.001	<0.001	<0.001
VIII Garden Fruits	0.002	0.002	<0.001	<0.001	<0.001
IX Fruits	<0.002	0.002	0.003 0.002	<0.001	<0.001

¹ All values listed are results of single determinations. Therefore the uncertainty of the data due to counting error and radiochemistry is approximately $\pm 10\%$. The data were acquired by J. T. Tanner and M. H. Friedman of this laboratory. "Upper limits" represent twice the standard deviation of the background count.

² Oils (X), Sugar adjunct (XI) and Beverages (XII) are not readily freeze dried; therefore they are not suitable for neutron activation analysis at this time.

³ USDA (9) regions.

Levels of Mirex and Some Other Organochlorine Residues in Seafood from Atlantic and Gulf Coastal States

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ABSTRACT

A seafood monitoring program for the insecticide mirex was set up in 1971. Seventy-seven composite samples of oysters, crabs, shrimp, fish, and fish products were collected from seven locations within the area where mirex is used and from two check locations outside the treatment areas. The study showed that mirex occurred in only nine of the 77 samples (0.005-0.024 ppm range), all from near Savannah, Georgia. DDT or its metabolites occurred in 74 samples (0.002-2.475 ppm range). The polychlorinated biphenyl (PCB) Aroclor 1260 occurred in 46 samples; if extensive new methods of cleanup had not been used, these PCB residues probably would have been interpreted as mirex. Several early studies reporting extensive residues of mirex in marine life were made before the possibility of confusing Aroclor 1260 with mirex had been realized; hence these earlier studies may be in error.

Introduction

Throughout a nine-State region in the Southeastern United States, baits containing the insecticide mirex are the main method of controlling the imported fire ant, an important agricultural pest and a nuisance to humans. At least 95% of the mirex used in the United States as an insecticide is used to control this one pest; since 1967 more than 10 million acres a year have been treated with mirex (1). Since many of the areas with the heaviest infestation of the imported fire ant are in coastal regions, a considerable amount of the mirex used has been near the coastline of the Atlantic Ocean and the Gulf of Mexico.

Mirex is an organochlorine pesticide and like most other organochlorine pesticides has a long field life.

When applied at the normal rate, 1.7 g actual toxicant per acre, minute residues have been found in a wide variety of nontarget organisms in the treated areas (1).

In 1968 and 1969 the U.S. Department of Agriculture (USDA) conducted a study on Cat Island off the coast of Mississippi, and in 1969 and 1970 USDA cooperated with the U.S. Department of the Interior (USDI) at Charleston, S.C., in a similar study. The studies, both unpublished, showed residues ranging from a high of 2 ppm in some bottom-feeding fish immediately following a treatment (probably indicating that the fish had fed directly on the bait) to a low of 0.001 ppm. Small residues could be detected as much as 2 years later. These two studies were restricted to small bays or estuaries where mirex was applied directly to the water or to the immediately surrounding salt marsh. The USDA has not sampled fish and marine life from areas lying off treated sections of the coastline.

Other studies have indicated that mirex residues were being found well outside the immediate treatment area and were showing up in commercial catches of marine life destined for human consumption. Butler (2) reported mirex as one of the most abundant of the organochlorine pesticides found in shellfish off the Atlantic, Gulf, and Pacific coasts. In a survey of pesticides in blue crabs off the coast of South Carolina (3), North Carolina, Georgia, and Florida (4), 35% of the samples from the 20 collecting sites contained mirex residues with a mean of 0.076 ppm and extremes of 0.005 to 0.389 ppm. No similar monitoring for mirex has been conducted with marine fish, but the widespread occurrence of mirex in invertebrates indicates that larger predators of the same area could have higher residues of this pesticide.

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The occurrence of even small residues of mirex in crustaceans has taken on new significance with the discovery that mirex, like many other organochlorine pesticides, is lethal to juvenile shrimp, crabs (5), and juvenile crayfish (6).

The USDA, as part of an overall study of the distribution of mirex through the environment, initiated a preliminary survey for mirex in seafood in spring 1971. Results of this study are the subject of this report. Primary purpose of the study was to survey various types of seafood and marine products to determine how extensively mirex residues were occurring.

Sampling Procedures

The seafood analyzed in this study was collected from nine sampling areas shown in Figure 1. The areas were not chosen randomly. To assure finding residues for this preliminary study, researchers collected some samples from areas where mirex had been used extensively. Correspondingly, the first seven of the following sampling areas were set up in areas with a documented history of mirex use; sampling areas 8 and 9 were located outside mirex treatment areas. The following paragraphs describe specific areas.

(1) *Mississippi Sound:* This region lies between the Mississippi River Delta and the coast of Mississippi. It



FIGURE 1.—Mirex sampling areas, Atlantic and Gulf Coast States, 1971. Numbers 1 to 9 show sampling sites, shaded areas indicate heaviest use of mirex. Dotted line denotes approximate range of imported fire ant.

is fed by several rivers, the most notable of which is the Pearl River, and the drainage from Lake Pontchartrain in Louisiana. All the rivers drain areas which are heavily infested with the imported fire ant and which have been treated extensively with mirex. The coast of Harrison County was treated aerially in 1966 and 1969, and much of Hancock County was treated in 1969 and 1970; Cat and Ship Islands in Mississippi Sound received three applications of mirex in 1968 and 1969 in an experimental eradication trial.

(2) *Mobile Bay:* Mobile Bay, Alabama, has not been the site of any organized control programs. However, individual farmers have attempted to control the imported fire ant in the surrounding areas in one of the largest mirex programs in the Southeast. Most farmers in the area mix mirex bait with fertilizer which they apply to pastures every spring.

(3) *Tampa Bay:* Approximately 3 million acres surrounding Tampa Bay, Florida, including the counties of Pinellas, Hillsboro, Polk, Hardee, Sarasota, and Manatee, received two or three aerial applications of mirex from 1967 to 1969 as part of a Federal - State cooperative program. Since 1969 the headwaters of many of the rivers feeding this bay also drained areas where State-farmer control programs have been effective. Probably the second largest amount of mirex ever to be applied areas within the range of the imported fire ant.

(4) *Jacksonville, Florida:* Samples were collected from the mouth of St. John's River. Ants have never been too extensive in the area, but several control programs have been conducted in the past 5 years. It probably has received less mirex than any of the other six sampling areas within the range of the imported fire ant.

(5) *Savannah, Georgia:* Approximately 2.5 million acres of land in the area of Savannah, Georgia, and adjacent South Carolina received three complete blanket applications of mirex bait between the fall of 1967 and 1969. This was an experimental attempt to eradicate an isolated pocket of ants, and was followed in 1970 by smaller programs farther inland. The program included extensive treatment of the marsh areas along the coasts of South Carolina and Georgia. To date, this is probably the largest area of marsh, tidal flat, and open water which has been treated in the Southeast, and the total amount of mirex used since 1968 is probably the largest ever used in the United States. Residues of mirex from marine life in this area have been detected in routine monitoring conducted along with the eradication experiment.

(6) *Charleston, South Carolina:* A block of approximately 500,000 acres was treated in the Charleston area in the fall of 1969-70. The area treated stretched from Berkeley County through Dorchester, Charleston, and Colleton Counties. Most areas were treated at least twice.

However, the coastal area, a strip 3 to 12 miles wide between the treatment block and the coast, was not treated, in compliance with USDA policy on the use of mirex (7). The headwaters of numerous streams that drained treated areas did empty into the coastal strip, however, which included all coastal islands and beaches, tidal marshes, and major estuaries.

(7) *Morehead City, North Carolina*: A small isolated infestation of the imported fire ant near Morehead City in Carteret and Onslow Counties, North Carolina, is presently the point farthest north which is infested by this pest. Since 1963 parts of the area have been treated yearly to reduce the population of the ant.

(8) *Chesapeake Bay* and (9) *Delaware Bay*: These two sampling areas are located well beyond the present range of the imported fire ant and there is no reason to believe that mirex has been applied in either area. The two areas were chosen as check areas.

Field inspectors for the Animal and Plant Health Inspection Service, USDA, stationed in the nine areas, were assigned the task of collecting the necessary samples. Each inspector was furnished a list of desired samples and instructions for collecting, handling, and shipping them. Samples were usually purchased from commercial sources such as fishing boats, processing plants, or wholesale distribution points. Occasionally samples of game fish were obtained from sport fishermen. When possible, samples of the desired animals were obtained from three or more sources in each sampling area and combined to give a single composite sample. Types and sizes of samples obtained from each sampling area are given in Tables 1 and 2. Samples were wrapped in aluminum foil, frozen, and shipped to Gulfport, Mississippi, for analysis. At Gulfport they were rechecked and their identifications were verified. If the samples were too small or represented too few locations within a sampling area, inspectors were requested to furnish additional samples. Considerable effort was made to verify that the samples had come from the designated sampling area and had not been shipped or carried in from outside.

Analytical Procedures

Samples were thoroughly scrubbed to remove mud, algae, and other residues; they were ground whole and mixed in a Waring blender to make a composite sample. Samples were prepared and analyzed on a whole-body basis as received, except shellfish which were first shucked. Each sample of seafood usually consisted of a composite of five or more individuals, except the larger game fish. A 20-g subsample of the composite was removed and analyzed by our standard analytical method (1). Basically this consists of extracting with a mixture of hexane and isopropanol, and treating with concen-

trated sulfuric acid cleanup. The sulfuric acid destroys dieldrin, endrin, and organophosphorus insecticides, but the improvement in sensitivity by this cleanup was considered more than adequate compensation for the loss of these other pesticides. The final extract was cleaned up on a Florisil column and concentrated to the desired level for analysis. If polychlorinated biphenyls (PCB's) were suspected in the first analysis, their presence usually being indicated by a series of characteristic peaks, the sample was reprocessed to separate the PCB's from the pesticides. This technique is basically that described by Amour and Burke (8) but has been modified according to Gaul and Cruz-LaGrange (9) and our own laboratory (1).

After concentrating to the appropriate volume, the extraction from both methods of cleanup were chromatographed on a Hewlett-Packard Model 402 dual column gas chromatograph equipped with dual electron capture detection. Each sample was always analyzed on two different columns: the first column was a mixture of 1.5% OV-17 and 1.95% QF-1 on Gas Chrom Q. The temperatures of the injector, oven, and detector were 250°C, 200°C, and 210°C, respectively. The second column was 2% DC-200 Gas Chrom Q with injector, oven, and detector temperatures of 245°C, 175°C, and 205°C, respectively. Argon-methane at 80 ml/min was the carrier gas. Level of detection was 0.001 ppm for DDT and its metabolites, 0.005 ppm for mirex, and 0.01 ppm for the PCB, Aroclor 1260. Quantitative analysis for the PCB is still being developed, so the values presented should be considered tentative.

Results and Discussion

Table 2 presents the residue levels of mirex, DDT and its metabolites, and PCB Aroclor 1260 found in 77 seafood samples. DDT and its metabolites were recovered in 74 samples (range 0.002-2.475 ppm). The PCB Aroclor 1260 was found in 46 samples (range 0.01-1.17 ppm). Mirex was found in only 9 samples (range 0.007-0.024). All samples containing mirex were from around Savannah, Georgia, an area with a history of concentrated mirex use, among the most extensive in the United States. The recovery of mirex in only 12% of the samples, all from one area, could indicate that mirex is not so general nor widespread a contaminant of seafood as are PCB's and DDT. This does not correspond to earlier seafood studies (2,3,4) which reported that mirex occurred much more frequently and densely in many of these same collection sites.

Probably the reason for the discrepancy between this study and earlier studies is the confusion of Aroclor 1260 with mirex. The retention time for the last peaks of Aroclor 1260 is almost identical to the retention

time for mirex on most columns routinely used for analyzing mirex (1). Unless extensive additional cleanup procedures are employed, such as those used in this study, it is almost impossible to separate these two peaks. In this study, if the problem of PCB confusion had not been recognized and the special cleanup procedures used, the PCB peaks probably would have been reported as mirex. The possibility of confusing PCB's with the organochlorine pesticides was not generally recognized until 1970, and methods for distinguishing PCB's from mirex were not developed for general use until 1971. Since the three previous studies which reported mirex in seafood were all conducted before 1971, they probably did not recognize the problem and may have inadvertently reported the PCB Aroclor 1260 as mirex.

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TABLE 1.—Summary of seafood samples from Atlantic and Gulf Coastal sites

LOCATION	TYPE OF SEAFOOD ¹							
	CRABS	SHRIMPS	OYSTERS & SHELLFISH	SQUIDS	PREDATORS/ GAME FISH	BOTTOM FISH	HERRING	FISH PRODUCTS
1. Mississippi Sound	+	+	—	+	+	—	—	+
2. Mobile Bay	+	+	+	+	+	+	+	—
3. Tampa Bay	+	—	—	—	+	+	—	—
4. Jacksonville, Fla.	+	+	—	—	+	+	—	—
5. Savannah, Ga.	+	+	+	+	+	+	+	—
6. Charleston, S. C.	+	+	+	+	+	+	—	—
7. Morehead City, N. C.	+	+	+	—	+	+	—	+
8. Chesapeake Bay	+	—	—	—	—	—	+	—
9. Delaware Bay	+	—	—	—	+	+	—	—

¹ Plus sign (+) denotes sample collected minus sign (—) denotes no sample collected.

TABLE 2.—Mirex, DDT and metabolites, and PCB residues in seafood^a

SPECIES	SAMPLE QUANTITY	RESIDUES IN PPM					
		DDE	TDE	DDT	TOTAL DDT & METABOLITES	AROCLOR 1260	MIREX
SAMPLING AREA 1—MISSISSIPPI SOUND							
Crabs	15	0.102	0.024	0.020	0.146	0.07	—
Shrimp	1 kg	0.004	—	0.003	0.007	—	—
Squid	1 kg	0.001	0.009	0.007	0.017	0.03	—
Flounder	3	0.031	0.007	0.009	0.047	0.03	—
Speckled sea trout	3	0.100	0.040	0.060	0.200	0.20	—
Spanish mackerel	3	0.095	0.026	0.040	0.161	0.06	—
Weak fish	5	0.049	0.010	0.050	0.109	0.12	—
Atlantic whiting	5	0.070	—	0.020	0.090	0.10	—
Fish oil	1 liter	0.070	0.030	0.020	0.120	0.02	—
Fish meal	1 kg	0.030	0.016	0.011	0.057	0.02	—
Ground fish (unprocessed)	1 kg	0.120	0.030	0.027	0.177	0.04	—
SAMPLING AREA 2—MOBILE BAY, ALA.							
Blue crabs	15	0.157	0.040	0.037	0.234	0.12	—
Shrimp	1.3 kg	0.006	0.002	0.003	0.011	0.01	—
Oysters	20+	0.042	0.004	0.003	0.049	0.03	—
Squid	10	—	—	—	—	0.04	—
Flounder	3	0.085	0.017	0.036	0.138	0.11	—
Speckled sea trout	1	0.710	0.170	0.280	1.160	0.40	—
Spanish mackerel	2	0.020	0.008	0.014	0.042	0.18	—

TABLE 2.—Mirex, DDT and metabolites, and PCB residues in seafood¹—Continued

SPECIES	SAMPLE QUANTITY	RESIDUES IN PPM					
		DDE	TDE	DDT	TOTAL DDT & METABOLITES	AROCLOR 1260	MIREX
Weak fish	5	0.297	0.078	0.127	0.502	0.37	—
Atlantic whiting	5	0.052	0.010	0.008	0.070	0.11	—
Croaker	3	0.164	0.038	0.034	0.236	0.33	—
Red snapper	2	0.023	—	0.063	0.086	0.14	—
Mullet	5	0.260	0.150	0.111	0.521	0.11	—
Anchovy	>20	0.044	0.050	0.033	0.127	0.08	—
Shad	>10	0.669	0.030	0.045	0.744	0.09	—
SAMPLING AREA 3—TAMPA BAY, FLA.							
Crabs	10	0.027	0.004	0.014	0.045	0.03	—
Weak fish	5	0.075	0.033	0.082	0.190	0.83	—
Mullet	5	0.071	0.051	0.045	0.167	0.31	—
SAMPLING AREA 4—JACKSONVILLE, FLA.							
Blue crabs	15	0.011	0.011	0.035	0.057	0.08	—
Shrimp	1 kg	0.006	0.014	0.029	0.049	0.60	—
Flounder	3	0.162	0.128	0.524	0.814	0.65	—
Weak fish	5	0.067	0.019	0.127	0.213	0.44	—
Gray snapper	2	0.007	—	—	0.007	—	—
Grouper	3	0.041	—	—	0.041	—	—
Drum	5	0.013	0.011	0.041	0.065	0.17	—
Croaker	5	0.015	—	—	0.015	—	—
SAMPLING AREA 5—SAVANNAH, GA.							
Blue crabs	16	0.023	—	—	0.023	—	0.014
Shrimp	0.5 kg	0.005	—	—	0.005	—	0.007
Pilot shrimp	0.5 kg	0.016	—	0.011	0.027	—	0.007
Oysters	15	0.009	—	—	0.009	—	—
Razorback clams	28	0.020	0.016	0.020	0.056	—	—
Squid	5	—	—	—	—	—	0.007
Flounder	4	0.014	—	0.004	0.018	—	0.005
Weak fish	1	0.020	0.006	0.014	0.040	—	0.024
Mullet	1	0.004	—	0.003	0.007	—	0.006
Shad	13	0.027	0.023	0.028	0.078	—	0.009
Other fish	5	0.041	0.032	0.034	0.107	—	0.014
SAMPLING AREA 6—CHARLESTON, S. C.							
Blue crabs	10	0.057	—	—	0.057	—	—
Shrimp	1 kg	0.015	—	—	0.015	0.02	—
Oysters	>15	0.017	—	—	0.017	—	—
Squid	>15	—	—	—	—	—	—
Croaker	1	0.042	0.008	0.004	0.054	—	—
Mullet	5	0.027	0.017	0.004	0.048	—	—
SAMPLING AREA 7—MOREHEAD CITY, N. C.							
Blue crabs	15	0.012	—	0.001	0.013	—	—
Shrimp	1 kg	0.001	—	0.001	0.002	—	—
Oysters	24	0.039	0.017	0.006	0.052	0.02	—
Clams	20	0.003	0.002	0.003	0.008	—	—
Scallops	12	0.002	—	0.006	0.010	—	—
Flounder	3	0.030	—	0.010	0.040	—	—
Spanish mackerel	2	0.050	—	0.060	0.110	—	—
Weak fish	2	0.080	—	0.120	0.200	—	—
Blue fish	2	0.132	—	0.101	0.233	—	—
Mullet	5	0.115	0.082	0.189	0.386	—	—
Other	10	0.010	0.004	0.008	0.022	—	—
Fish meal	0.5 kg	0.095	0.050	0.060	0.205	0.11	—
SAMPLING AREA 8—CHESAPEAKE BAY							
Blue crabs	25	0.019	0.011	0.012	0.042	0.02	—
Weak fish	3	0.459	0.177	0.172	0.798	0.49	—
Striped sea bass	3	0.027	0.005	0.011	0.043	0.02	—
Spot	5	0.017	0.018	0.028	0.053	0.10	—
Herring	10	0.006	0.006	0.006	0.018	—	—
SAMPLING AREA 9—DELAWARE BAY							
Blue crabs	15	0.128	0.056	0.046	0.230	0.13	—
Flounder	2	0.023	—	0.021	0.044	0.07	—
Weak fish	5	0.479	0.059	0.044	0.582	0.23	—
Striped bass	2	0.046	0.007	0.033	0.086	0.07	—
Mullet	5	0.800	0.180	0.120	1.100	0.21	—
Other	2	1.295	0.550	0.630	2.475	1.17	—
Croaker	1	0.060	0.030	0.060	0.150	0.50	—

¹ Minus sign (—) denotes no residues found at lowest level of detection; 0.001 ppm for DDT, 0.005 ppm for Mirex, 0.01 for Aroclor 1260.

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Mirex Residues in Selected Estuaries of South Carolina—June 1972¹

P. W. Borthwick,² G. H. Cook,² and J. M. Patrick, Jr.²

ABSTRACT

Estuarine sediments, crabs, shrimps, and fishes were collected in June 1972 at eleven stations two years after aerial applications of mirex bait for control of fire ants in coastal areas near Charleston, S.C. These stations had previously been monitored (October 1969 to June 1971) when levels of mirex in animal samples were: crabs, 0-0.60 ppm; shrimps, 0-1.3 ppm; and fishes, 0-0.82 ppm.

The recent study showed that mirex was present in three species of fishes (white catfish, 0.021 ppm; bluegill, 0.047 ppm; carp, 0.12 ppm) and blue crabs (0.026 ppm) at two freshwater stations. However, mirex was not detected in 36 animal samples, most of which were taken from nine saline stations in the estuaries after a period of restricted use of the pesticide. Analysis of bottom sediment samples at all stations detected no mirex. The lower limit of detection for mirex was 0.01 ppm.

Introduction

In June 1972 samples of estuarine sediments, crabs, shrimps, and fishes were collected at 11 stations near Charleston, S.C., where mirex fire ant bait had been applied aerially to coastal areas from October 1969 to December 1970. The United States Department of Agriculture supervised two applications of mirex, by fixed-wing aircraft, to several hundred thousand acres in the Charleston area. Treatments were terminated approximately 24 months before the June 1972 collec-

tions. However, 18 months lapsed since special applications were made by helicopter to 1200 acres at Toogoodoo Creek: Stations A, B, C, and D; and by hand seeder around a one-acre pond at Riverland Terrace: Station 1 (Fig. 1).

Since 1970 less extensive applications have been made for control of nuisance populations of fire ants. During the 1971-72 cooperative Federal State Control Program, mirex bait was applied aerially in South Carolina and seven other Southeastern states at property owners' requests.

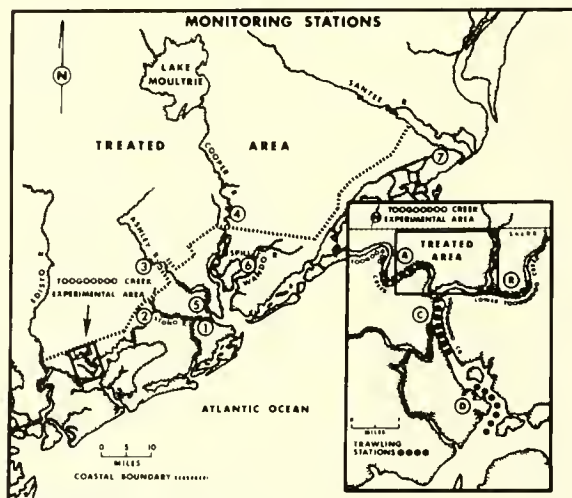


FIGURE 1—Map of study area showing location of mirex sampling sites, South Carolina—June 1972

¹ Contribution No. 168 from the Gulf Breeze Environmental Research Laboratory, United States Environmental Protection Agency, Gulf Breeze, Florida 32561; Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon.

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The use of mirex decreased when the U.S. Environmental Protection Agency (U.S. EPA) issued orders (1, 2) to cancel the registration of products containing mirex, pending relabeling. The orders required that no mirex be applied aerially near estuaries and other aquatic areas, wildlife refuges, or heavily forested areas. The present study was implemented to determine how much mirex remained in the estuarine fishes and crustaceans following a period of restricted use of the pesticide.

The stations (Fig. 1) and analytical techniques were identical to those used in a more comprehensive study of the area (3). Samples were collected and analyzed by the United States Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida.

Results and Discussion

In June 1972, 24 months after large-scale aerial treatment of inland areas, mirex residues greater than 0.01 ppm were found in only 4 of 40 animal samples (Table 1). All four samples were collected at the two freshwater stations located on the Ashley and Cooper Rivers (Stations 3 and 4, Fig. 1) which drain watersheds within the treated area. Three species of freshwater fishes and blue crabs contained the following amounts of mirex (ppm):

white catfish	0.021
bluegill	0.047
carp	0.12
blue crabs	0.026

Except for the bluegill, these animals are omnivorous bottom-dwellers. Blue crabs are euryhaline; they occasionally enter brackish and fresh waters of estuaries.

Mirex was not detected (Table 1) in the remaining 36 animal samples, most of which were taken at 9 stations located on tidal creeks in salt marsh areas that support populations of finfish and crustaceans. Many of these animals are transient and spend only a portion of their lives in the estuary. No mirex was detected in bottom sediments sampled at each location.

The lower limit of detection for mirex with the method employed (3) was 0.01 ppm.

Between October 1969 and June 1971 mirex residues in economically important members of the estuarine food chain varied as follows: crabs, 0-0.60 ppm; shrimps, 0-1.3 ppm; and fishes, 0-0.82 ppm. Levels of mirex in these species diminished to less than 0.01 ppm over a period of 18 to 24 months after the last aerial broadcast treatments to coastal South Carolina.

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TABLE 1.—Whole body mirex residues, ppm, in estuarine animals, and sediments of South Carolina—June 1972

STATION LOCATION		TOOGOOD CREEK				RIVER- LAND TERRACE POND	STONO RIVER	UPPER ASHLEY RIVER	COOPER ² RIVER	LOWER ASHLEY RIVER	WANDO RIVER	SOUTH SANTEE RIVER
SPECIES	STATION IDENTIFICATION	A	B	C	D	1	2	3	4	5	6	7
CRABS												
<i>Callinectes sapidus</i> (blue crab)		— ¹	—	—	—	—	—	—	0.026	—	—	—
<i>Uca pugilator</i> (sand fiddler)				—								
SHRIMPS												
<i>Penaeus aztecus</i> (brown shrimp)		—	—	—	—	—	—	—	—	—	—	—
<i>Palaemonetes pugio</i> (grass shrimp)						—						
FISHES												
<i>Leiostomus xanthurus</i> (spot)		—		—	—	—	—	—	—	—	—	—
<i>Bairdiella chrysura</i> (silver perch)			—									—
<i>Ictalurus catus</i> (white catfish)								0.021				
<i>Cyprinus carpio</i> (carp)									0.12			
<i>Lepomis macrochirus</i> (bluegill)								0.047				
MOLLUSKS												
<i>Crassostrea virginica</i> (oyster)				—								
<i>Mercenaria mercenaria</i> (hard clam)									—			
SEDIMENT												
		—	—	—	—	—	—	—	—	—	—	—

¹ — indicates <0.01 ppm mirex.

² Freshwater stations.

Monitoring 2,4-D Residues at Loxahatchee National Wildlife Refuge

Donald P. Schultz¹ and Eugene W. Whitney²

ABSTRACT

Over 7,000 acres along the Hillsboro perimeter canal in Loxahatchee National Wildlife Refuge (NWR), Florida, were sprayed in 1971 with the dodecyl-tetradecyl amine salts of 2,4-D (DTA-2,4-D) at a rate of 4.48 kg acid equivalent (a.e.) per hectare to control waterhyacinth (*Eichornia crassipes*). Three stations were established along the canal to collect samples of fish, water, and mud for residue analysis and to monitor water quality.

The initial application of 2,4-D, followed by spot treatments of DTA-2,4-D and/or the dimethylamine salt of 2,4-D (DMA-2,4-D) over a 4-month period, achieved excellent control of waterhyacinth. The highest 2,4-D residue level measured in water (0.037 mg/l) was found on the day following the initial treatment. The highest levels in hydrosol (0.005 mg/kg) occurred 3 to 15 days after treatment. Among 60 samples of fish, 3 had herbicide residue levels greater than 0.010 mg/kg, 16 had less than 0.010 mg/kg, and the remainder had no detectable residue levels. Breast muscle and liver of common Florida gallinules (*Gallinula chloropus*) had residue levels of 0.30 mg/kg and 0.675 mg/kg, respectively, 1 day after spraying, and no detectable residues 4 days after spraying. The herbicide apparently caused no ill effects on hatching of boat-tailed grackle (*Cassidix mexicanus*) eggs or development of fledglings.

Introduction

The Loxahatchee National Wildlife Refuge, located in southern Florida, consists of 143,000 acres of wetlands in the Florida Everglades. It is encircled by a 56-mile dike, and serves as a freshwater storage impoundment in the intricate water control system operated by the U.S. Army Corps of Engineers and South Florida Flood

Control District. Hillsboro Canal, a perimeter canal which was created by excavation for dike fill, parallels the inner side of the dike. It contains open water all year, and is used extensively for sport fishing. The refuge supports over 180 species of birds and is important in the preservation of such endangered species as the Florida Everglade kite (*Rostrhamus sociabilis*), great white heron (*Ardea occidentalis*), and American alligator (*Alligator mississippiensis*).

Over the past several years, waterhyacinth (*Eichornia crassipes*) has become entrenched to varying degrees in the canal. There have been numerous occasions when the canal was completely blocked by the plant, thus curtailing public use of the Refuge for fishing, waterfowl hunting, and bird watching. In addition, a small amount of feeding habitat for the Florida Everglade kite was lost since the kite requires open water to see and capture the snail which is the mainstay of its diet.

In order to maintain the desirable features of the Refuge, the Division of Refuges, Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior, decided in 1971 to control the waterhyacinth in the perimeter canal and its proximity by spraying the hyacinth with the dodecyl-tetradecyl amine salts of 2,4-D (Emulsamine-3, Amchem Products, Inc., Ambler, Pa.) at the recommended rate of 4.48 kg acid equivalent (a.e.) per hectare (4 lb/acre) (John Gallagher, Amchem Products, Inc., Ambler, Pa. 1971. *Personal communication*).

Mullison (1) has reviewed many articles dealing with the toxicity of 2,4-D to aquatic organisms and levels of 2,4-D residues in aquatic environments. However, no published reports are available pertaining to the DTA-2,4-D formulation. Therefore, a monitoring program was designed to obtain data that would support registration of DTA-2,4-D for use on waterhyacinths at Loxahatch-

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² Deceased. Formerly with Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior, University of Georgia, Athens, Georgia 30601.

ee NWR. This program was designed to obtain residue data from fish, water, and hydrosol, and to visually assess possible effects of the herbicidal applications on certain birds found in the Refuge.

Methods and Materials

Most of the herbicide was applied June 16-17 by a helicopter equipped with a microfoil boom. Spot treatments by airboat or helicopter were completed by October 15 to eliminate hyacinths that had not been killed in June. When the airboat was used, the 2,4-D formulation was the dimethylamine salt as opposed to the dodecyl-tetradecyl amine salts used in helicopter applications.

Three stations approximately 3 miles apart were established along the Hillsboro Canal (Fig. 1). Pretreatment samples of water, mud, and fish were taken in May 1971. Samples of fish, water, and mud for residue analysis were taken from each station at regular intervals after spraying.

Fish were obtained by gill netting. Fish samples were wrapped in aluminum foil, placed in plastic bags, frozen on dry ice, and kept frozen until residue analyses could be performed.

Water samples were a composite of shallow, medium, and deep water from each station. The samples were put in quart jars and acidified to about pH 1.5 with concentrated sulfuric acid; jars were capped with aluminum foil and screw caps. Hydrosol samples were a composite of three samples from each station, and were obtained with an Ekman dredge. These samples were placed in plastic bags, frozen on dry ice, and kept frozen until analyzed.

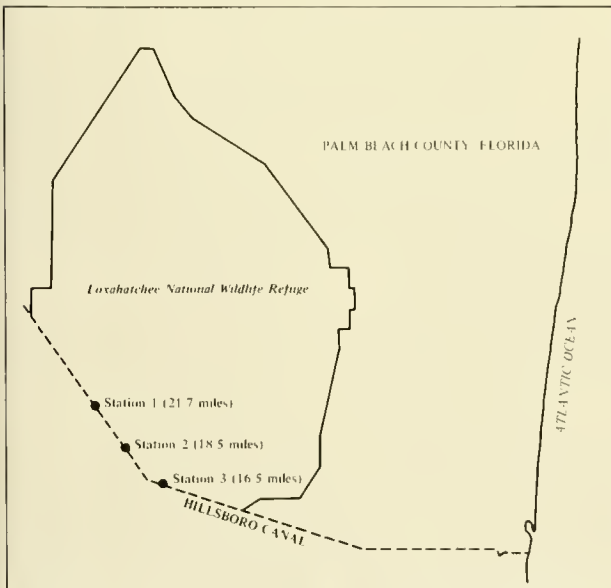


FIGURE 1.—Location of sampling stations at Loxahatchee National Wildlife Refuge.

Florida gallinules were killed by shotgun on six different occasions: once before initial treatment and five times afterward. All but one of the six samples consisted of three birds. The birds were wrapped in aluminum foil, bagged, and frozen until analyses were performed.

Six grackle nests containing eggs or young were located and permanently marked before spraying began. All the nests were in one colony in reeds immediately adjacent to a large clump of hyacinths, and were directly sprayed during treatment. Observations were made on hatching success and fledgling mortality for 6 weeks after spraying.

Dissolved oxygen content, pH, temperature, and water-hardness were determined by standard chemical or physical techniques (2).

Residue determination procedures for water, hydrosol, fish, and gallinules are given in Supplements A, B, C, and D. All solvents used were glass-distilled. Results were not corrected for percent recovery.

Results and Discussion

Physical and chemical parameters of the water and the quantities of 2,4-D residues found in the water and hydrosol are listed in Table 1. Fluctuations in dissolved oxygen content and pH could not be correlated with herbicidal application, and may have resulted from diurnal variations and the bloom and decay of algae.

The highest 2,4-D residue level in water was 0.037 mg/liter found 1 day after the initial treatment at Station 2. Water from Station 1 contained 0.016 mg/liter 1 day after treatment, but all other samples contained less than 0.010 mg/liter. There have been no published reports of DTA-2,4-D residues in water or hydrosol. Smith and Isom (3) found residues of 0.068 mg/liter of the butoxyethanol ester of 2,4-D (BEE-2,4-D) 8 hours after treatment at 40 to 100 lb of 2,4-D a.e. per acre in a Tennessee Valley Authority reservoir. Wojtalik et al. (4) found residues of 1.8 mg/liter 24 hours after application of 40 lb a.e. per acre of DMA-2,4-D. Both reports dealt with treatment of a submersed aquatic plant (*Myriophyllum spicatum*).

In our study, the target was thick mats of floating waterhyacinths. These plants intercepted much of the herbicide that otherwise would have entered the water. This interception partly explains the low residue levels found in water.

Only three hydrosol samples from Loxahatchee NWR contained detectable levels of 2,4-D (Table 1). None of these residues exceeded 0.005 mg/kg. In contrast, Smith and Isom (3) reported residues of 56 mg/kg BEE-2,4-D 96 hours after treatment at one sampling station. As with residue levels in water, absorption of the herbicide by the waterhyacinth mat was probably one reason we found such low residues in hydrosol.

TABLE 1.—Analyses of water and hydrosol samples from Loxahatchee National Wildlife Refuge

STATION	DATE	WATER ¹						HYDROSOL ²
		DAYS AFTER TREATMENT	TEMP, °C	DISSOLVED OXYGEN, MG/L	PH	TOTAL HARDNESS, MG/L	2,4-D, MG/L	2,4-D, MG/KG
1	4-27-71	0	26	5.6	7.3	200	0.000	0.000
1	6-17-71	1	30	8.0	7.4	250	0.016	0.000
1	6-19-71	3	27	7.9	7.6	250	<0.001	<0.005
1	6-20-71	4	— ³	—	—	—	<0.001	<0.005
1	6-24-71	8	27	9.0	7.6	240	0.008	—
1	7-1-71	15	30	11.0	7.4	250	0.002	0.000
1	7-15-71	29	30	6.0	8.2	250	0.002	0.000
1	8-12-71	57	27	6.0	7.8	270	0.004	0.000
1	10-7-71	113	28	8.0	7.5	360	—	0.000
2	6-18-71	1	—	—	—	—	0.003	0.000
2	6-20-71	3	28	7.2	7.8	230	—	0.000
2	6-24-71	7	—	—	—	—	<0.001	0.000
2	7-2-71	15	29	11.0	7.4	240	<0.001	<0.005
2	7-15-71	28	31	6.0	8.2	240	<0.001	0.000
2	8-12-71	56	28	6.0	7.8	270	0.001	0.000
2	10-7-71	112	28	5.0	7.3	300	—	0.000
3	4-28-71	0	30	7.5	7.0	230	—	0.000
3	6-18-71	1	31	9.5	7.6	280	0.000	0.000
3	6-20-71	3	29	8.7	7.9	240	0.001	—
3	6-24-71	7	28	7.4	7.8	280	0.007	0.000
3	7-2-71	15	29	9.0	7.4	260	0.002	0.000
3	7-15-71	28	32	8.0	8.2	260	0.002	0.000
3	8-12-71	56	29	12.0	8.0	270	<0.001	0.000
3	10-7-71	112	30	8.0	7.6	300	—	0.000

¹ 250 ml water used for analysis through 8 days; 500 ml thereafter.² 70 g hydrosol used for all analyses.³ Dashed line denotes no sample.

Other factors which may affect residue levels are photodecomposition and metabolism of the herbicide. For example, Aly and Faust (5) reported a photodecomposition loss of 50% of a sodium salt of 2,4-D in 50 minutes at pH 7.0. Also, Loos (6) and Menzie (7) reported in their reviews of 2,4-D metabolism that many microorganisms are capable of degrading 2,4-D. Hence, the disappearance of 2,4-D residues from water and hydrosol at Loxahatchee NWR probably was due to absorption, photolysis, and metabolism of the herbicide.

Data regarding 2,4-D residues in various sizes and species of fish and the station where fish were collected are given in Table 2. Usually, fish of the same species from the same collecting date and station were composited for residue analysis. In two instances each fish was analyzed individually. Muscle tissue, which included small quantities of bones, skin, or scales, was analyzed for 2,4-D.

Sixty analyses were made using a total of 193 fish. Only 19 samples contained detectable 2,4-D residues. Of these 19 samples, 8 had residues less than 0.010 mg/kg; 7 had between 0.010 and 0.050 mg/kg; 1 had between 0.051 and 0.10 mg/kg; and 3 samples had residues greater than 0.10 mg/kg.

With one exception, all fish samples which contained residues of 2,4-D were collected from a station which had been sprayed within the preceding 3 days (Tables 2, 3).

Smith and Isom (3) reported that one sample of bluegills (*Lepomis macrochirus*) collected 50 days after treatment of a reservoir at 100 lb BEE-2,4-D a.e./acre contained a residue of 0.15 mg/kg. The remainder of their fish samples contained no residues or less than 0.14

mg/kg. Wojtalik et al. (4) reported residues in gizzard shad (*Dorosoma cepedianum*) of 0.34 mg/kg 4 weeks after treatment and 0.22 mg/kg 3 months after treatment at 40 lb DMA-2,4-D a.e./acre. All other posttreatment samples contained less than 0.010 mg/kg 2,4-D residue. Based on our results and other studies (3,4), fish do not accumulate high levels of 2,4-D residues regardless of the formulation and concentration applied.

Physical and residue data from Florida gallinules are shown in Table 4. Levels of 2,4-D in breast muscle and liver in birds harvested 1 day after the initial spraying were 0.30 mg/kg and 0.675 mg/kg, respectively, but dropped to nondetectable levels 4 days after spraying; this implies that these birds excrete or metabolize the herbicide rapidly.

Three of the grackle nests sprayed contained eggs, and three contained nestlings. Subsequent observations showed that 10 of 11 eggs hatched, and that only 1 of 19 nestlings did not reach maturity. One of the nests which contained nestlings when observations began contained a new clutch of eggs 6 weeks later. Apparently, the herbicide had no effect on hatching of eggs or maturation of grackles.

Treatment of the Hillsboro Canal with 2,4-D resulted in excellent control of waterhyacinths. In July 1972 only a few scattered patches of hyacinths containing 10-50 plants each were observed in the canal.

In spite of the repeated applications of herbicide (Table 3), no adverse effects from the herbicide applications were noted in water quality, fish, gallinules, or grackles. Residue levels of the herbicide were very low in water and hydrosol, and dissipated rapidly from gallinule breast muscle and liver, and appeared to have no effect on grackle nestlings or hatching of eggs.

TABLE 2.—Residues of 2,4-D in fish from Loxahatchee National Wildlife Refuge

STATION	DATE	SPECIES	LENGTH, CM	WEIGHT, G	RESIDUES OF 2,4-D, MG/KG ¹	STATION	DATE	SPECIES	LENGTH, CM	WEIGHT, G	RESIDUES OF 2,4-D, MG/KG ¹
1	4-27-71	Redear sunfish (<i>Lepomis microlophus</i>)	17.8 15.2	91 45	0.000	3	6-20-71	Gar	38.1 33.0 63.5	227 136 1090	0.022
1	4-28-71	Largemouth bass (<i>Micropterus salmoides</i>)	28.0 28.0	227 228	0.000				45.7 43.2	368 368	
1	4-28-71	Brown bullhead (<i>Ictalurus nebulosus</i>)	30.5 25.4	454 227	0.000	1	6-20-71	Redear sunfish	20.3	—	0.000
1	4-28-71	Gar (<i>Lepisosteus spp.</i>)	35.6 33.0 30.5 38.1 48.3	182 136 91 182 454	0.000	3	6-24-71	Gar	38.1 35.6	590 545	0.000
3	4-28-71	Chubsuckers (<i>Erimyzon spp.</i>)	33.0 27.9	368 272	0.000	3	6-24-71	Chubsuckers	27.9 30.5 25.4 25.4 27.9	368 409 318 318 368	0.000 0.000 0.000 0.000 0.000
3	4-28-71	Largemouth bass	27.9 25.4	272 182	0.000	1	7-1-71	Redear sunfish	21.5 17.8	227 113	0.022
3	4-28-71	Redear sunfish	20.3 20.3 17.8	227 227 136	0.000	2	7-2-71	Chubsuckers	33.0 30.5	499 454	0.000
1	6-17-71	Redear sunfish	17.8 17.8 17.8 17.8	91 91 91 91	0.101	2	7-2-71	Redear sunfish	20.3 25.4	182 272	0.000
1	6-17-71	Brown bullhead	27.9 25.4 30.5 22.9 22.9	343 227 454 182 182	0.012	2	7-2-71	Bluegill (<i>Lepomis macrochirus</i>)	22.9 20.3 22.9 20.3 20.3	182 182 227 182 227	0.000
2	6-18-71	Chubsuckers	30.5 25.4 25.4 27.9 22.9	454 227 227 272 91	0.000	3	7-2-71	Redear sunfish	20.3 20.3 20.3	182 182 182	0.010
2	6-18-71	Gar	45.7 38.1 45.7 33.0	368 136 368 91	0.000	3	7-2-71	Chubsuckers	33.0	454	<0.010
2	6-18-71	Redear sunfish	35.6 20.3 17.8 17.8 22.9	182 182 136 91 272	0.000	1	7-15-71	Bluegill	20.3 20.3 18.9 18.9 22.9	136 227 113 113 294	0.000
3	6-18-71	Chubsuckers	33.0 33.0 30.5 27.9 27.9	590 454 409 227 227	<0.010	2	7-15-71	Chubsuckers	30.5 33.0	454 567	0.000
3	6-18-71	Redear sunfish	22.9 22.9 20.3 17.8 17.8	182 182 136 91 91	0.000	2	7-15-71	Bluegill	17.8 17.8 20.3 20.3 17.8	227 204 227 227 204	<0.010
3	6-18-71	Largemouth bass	35.6 27.9 27.9 27.9	454 318 318 318	0.000	3	7-15-71	Bluegill	20.3 20.3 20.3 17.8 20.3	227 227 227 113 227	0.000
1	6-19-71	Gar	40.6 48.3 55.9 33.0	227 454 454 136	0.000	1	8-12-71	Gar	38.1 48.3 43.2	136 454 272	0.106
1	6-19-71	Redear sunfish	22.9 20.3	227 136	<0.010	1	8-12-71	Brown bullhead	38.1 30.5 27.9	681 318 272	<0.010
1	6-19-71	Brown bullhead	25.4 25.4 27.9	227 227 318	0.000 0.000 0.000	1	8-12-71	Redear sunfish	17.8 17.8 17.8 17.8	91 91 91 91	<0.010
2	6-20-71	Redear sunfish	22.9 15.2 20.3	272 46 136	0.000	1	8-12-71	Chubsuckers	25.4 27.9 27.9 25.4 27.9	227 272 318 227 318	<0.010
2	6-20-71	Gar	55.9 40.6 48.3 38.1	727 227 368 227	0.028	2	8-12-71	Redear sunfish	22.9 22.9 17.8 22.9	227 227 91 227	0.162
2	6-20-71	Brown bullhead	27.9 25.4 27.9	272 227 227	0.000	3	8-12-71	Chubsuckers	30.5 33.0 25.4 27.9 25.4	454 567 227 272 227	0.000
3	6-20-71	Largemouth bass	27.9 25.4 25.4	227 227 272	0.000	3	8-12-71	Largemouth bass	27.9 27.9 27.9 30.5	227 272 368 368	0.012
3	6-20-71	Chubsuckers	25.4 27.9 25.4 22.9 25.4 25.4	272 272 272 182 227 227	0.000	3	8-12-71	Redear sunfish	20.3 20.3 22.9 17.8 17.8	227 227 368 136 136	0.024
						1	10-7-71	Brown bullhead	30.5 34.3 35.6 38.1	368 545 681 681	0.000

TABLE 2.—Residues of 2,4-D in fish from Loxahatchee National Wildlife Refuge—Continued

STATION	DATE	SPECIES	LENGTH, CM	WEIGHT, G	RESIDUES OF 2,4-D, MG/KG ¹
1	10-7-71	Gar	63.5	1362	0.000
			66.1	1544	
1	10-7-71	Redear sunfish	20.5	227	0.000
			20.3	182	
			20.3	227	
			19.1	182	
			24.2	368	
2	10-7-71	Redear sunfish	22.3	227	<0.010
			21.6	227	
			21.6	227	
			22.9	318	
			22.0	182	
2	10-7-71	Chubsuckers	36.9	726	0.000
			35.6	681	
			26.7	272	
			34.3	636	
			35.6	681	
3	10-7-71	Largemouth bass	35.6	636	0.000
			30.5	409	
			29.2	318	
			30.5	409	
			33.0	590	
3	10-7-71	Redear sunfish	22.9	227	0.070
			22.9	227	
			21.6	227	
			21.6	227	
3	10-7-71	Chubsuckers	33.0	545	0.000
			35.6	772	
			33.0	590	
			33.0	636	

¹ When only one set of data is given for a series of fish, the fish were composited for analysis.

TABLE 3.—Spray schedule for Loxahatchee National Wildlife Refuge¹

DATE	AREA SPRAYED	METHOD ²	ACREAGE
6-1-71	Vicinity of Station 3	Boat	8
6-2-71	Stations 1, 2, 3	Boat	4
6-16-71	Station 1	Air	53.6
6-17-71	Stations 2, 3	Air	105.0
6-18-71	Between Stations 1 and 2	Air	105.0
6-21-71	Vicinity of Station 1	Boat	1.5
6-22-71	Vicinity of Station 1	Boat	4.5
6-22-71	Vicinity of Station 1	Air	84.2
6-23-71	Station 2	Air	105.0
7-1-71	Station 3	Boat	10
7-2-71	Vicinity of Station 3	Boat	6.0
7-9-71	Vicinity of Station 3	Boat	8.0
7-12-71	Stations 2 and 3	Boat	10.0
7-26-71	Stations 1 and 2	Air	95.0
7-27-71	Station 2	Air	105.0
7-28-71	Vicinity of Station 3	Boat	13.5
8-3-71	Vicinity of Station 1	Boat	12.0
8-3-71	Vicinity of Station 2	Air	23.3
8-4-71	Station 2	Air	105.0
8-4-71	Vicinity of Station 1	Boat	9.0
8-5-71	Between Stations 2 and 3	Boat	12.0
8-5-71	Vicinity of Stations 1-3 (flats)	Air	105.0
8-6-71	Station 1 (flats)	Air	105.0
8-6-71	Between Stations 2 and 3	Boat	8.0
8-9-71	Between Stations 2 and 3	Boat	8.5
8-9-71	Station 2	Air	105.0
8-10-71	Station 2	Air	17.5
8-11-71	Stations 1-3 (flats)	Air	77.5
8-11-71	Vicinity of Station 1	Boat	7.5
8-12-71	Vicinity of Stations 1-3	Boat	6.0
8-12-71	Station 3	Boat	5.0
8-16-71	Vicinity of Station 2	Boat	6.0
8-17-71	Vicinity of Stations 1 and 2	Boat	3.0
10-7-71	Stations 1-3	Boat	6.0

¹ All herbicide was sprayed at the rate of 4 lb acid equivalent per acre.

² Herbicide applied by boat was the dimethylamine salt of 2,4-D. Aerial applications were the dodecyl-tetradecylamine salts of 2,4-D.

TABLE 4.—Residues of 2,4-D found in breast muscle and liver of common Florida gallinules (*Gallinula chloropus*)

HARVEST DATE ¹	WEIGHT OF BIRDS, G	COMBINED WEIGHT OF BREAST MUSCLE, G	RESIDUES OF 2,4-D, MG/KG	COMBINED WEIGHT OF LIVER, G	RESIDUES OF 2,4-D, MG/KG
6-18-71	363	98.8	0.000	19.4	0.000
	317				
	389				
6-18-71	390	105.1	0.300	22.9	0.675
	358				
	460				
6-21-71	357	66.0	0.000	11.2	0.000
	373				
	396				
6-23-71	320	75.4	0.000	15.5	0.000
	314				
	271				
6-25-71	261	53.7	0.000	9.5	0.000
	283				

¹ Control birds harvested 0.5 miles from site of spraying.

Supplement A

Method for analysis of water samples for determining concentrations of 2,4-D acid equivalent of Dodecyl-Tetradecyl amine salts or dimethylamine salt of 2,4-D.

1. Acidify water samples at time of collecting to a pH of less than 2 with concentrated sulfuric acid.
2. Extract representative sample of water (250-500 ml) three times with 100 ml chloroform.
3. Evaporate chloroform in a 500-ml round-bottom flask just to dryness with a rotary evaporator (do not allow water bath to exceed 45° C).
4. Add 5 ml diazomethane to the residue in flask. Allow to sit several minutes at room temperature. Add one-half ml benzene to flask and evaporate excess diazomethane in a hot water bath (60-70°C). Do not allow benzene to evaporate (8).
5. Add 5 ml benzene to flask. Transfer esterified 2,4-D to cleanup column.
6. Quantify on gas chromatography. Recovery from spiked samples was $97.5 \pm 2.5\%$.

Supplement B

Methods for analysis of hydrosol samples for determining concentrations of 2,4-D acid equivalent of dodecyl-tetradecyl amine salts or dimethylamine salt of 2,4-D.

1. Thaw samples. Place 70-g portion in stainless steel grinding flask.
2. Add the following mixture to the flask: 1.0 ml 20% sulfuric acid, 15 ml acetone, 25 ml petroleum ether, 100 ml diethyl ether, 10 g Celite 545 (Fisher Scientific, Inc.).
3. Grind mixture for 2 minutes at medium speed. Suction-filter liquid through Whatman #1 filter paper (use teflon cloth if hydrosol has high clay content).
4. Repeat procedure 2 and 3. Rinse flask and hydrosol with two 25-ml aliquots of diethyl ether:petroleum ether (1:1).

5. Pour filter flask contents into 1-liter separatory funnel. Rinse flask with 10-15 ml of diethyl ether: petroleum ether mixture and add to separatory funnel.
6. Partition filtrate with 100 ml 0.05 N NaOH. Check pH; if less than 10, add enough 6 N NaOH to bring pH to 10 or higher (9). Partition filtrate a second time with NaOH.
7. Acidify aqueous phase to a pH of less than 2 by dropwise addition of 20% H_2SO_4 (about 3 ml total).
8. Quantitatively transfer acidified aqueous phase to 1-liter separatory funnel.
9. Follow procedure as outlined for water samples beginning with step 2. Recovery from spiked samples was $90 \pm 2.5\%$.

Supplement C

Method for analysis of fish and gallinule samples for determining concentrations of 2,4-D acid equivalent of dodecyl-tetradecyl aminé salts or diethylamine salt of 2,4-D.

1. Thaw fish sufficiently to remove head and viscera; thaw gallinules sufficiently to remove breast muscle and liver. Chop tissue into small pieces (ca. one-half-inch cubes) and grind to a fine powder with twice their weight of dry ice in a Waring blender. Allow dry ice to sublime in a freezer (10).
2. Weight out portion of tissue powder (usually 25 g), mix with 100 g anhydrous Na_2SO_4 , and allow to stand for 45-60 minutes with occasional stirring until powder is dry.
3. Pack powder lightly in a glass column (22-mm inside diameter by 30 cm) fitted with a fritted glass disk. Elute column with 200 ml methanol:phosphoric acid (99:1, v/v) (11).
4. Place eluate in a 1-liter separatory funnel containing 500 ml deionized water, and adjust pH to 4.5.
5. Add 100 ml petroleum ether to separatory funnel and shake vigorously for 2 minutes. Draw off petroleum ether, and repeat the ether extraction two more times.
6. Acidify water-methanol mixture to a pH of 1.5 with concentrated H_2SO_4 . Extract mixture three times with 100-ml portions of diethyl ether.
7. Pass diethyl ether extract through glass column containing 12 cm anhydrous Na_2SO_4 , and collect ether in a 500-ml round-bottom flask.
8. Evaporate ether just to dryness on a rotary evaporator.
9. Follow procedure as outlined for water samples beginning with step 4. Recovery from spiked samples was $90 \pm 2.5\%$.

Note: if large quantities of fat or pigment are present in extracts, a preliminary extraction before step 4 may be done by putting the methanol-water extract into water adjusted to pH 8 with sodium bicarbonate and extract-

ing with petroleum ether. The aqueous extract may then be adjusted to pH 4.5 and extracted with petroleum ether.

Caution! There will be violent gas evolution at this stage.

Supplement D

Procedure for cleaning up 2,4-D acid residues derivatized with diazomethane.

1. Place glass-wool plug in bottom of glass column (22-mm inside diameter by 30 cm). Add 8 cm silica gel (Brinkman #7754, Brinkman Instruments, Inc., Cantiague Road, Westbury, New York 11590) and 2 cm anhydrous Na_2SO_4 .
2. Rinse column with 150 ml benzene. Discard eluate. Quantitatively transfer 2,4-D methyl ester from round-bottom flask to top of column.
3. Elute column with 180 ml of 2% diethyl ether in benzene. Collect eluate in porcelain casserole and evaporate to a small volume on a hot plate in a hood.
4. Transfer 2,4-D methyl ester to suitable container, and bring up to volume for analysis by gas chromatography.

The gas chromatograph used for the residue analyses was a Perkin-Elmer Model 800 equipped with a Tracor Electrolytic Conductivity Detector. The column was coiled glass, 6-ft-by-1/8-inch inside diameter, packed with 10% DC-200 on Gas-Chrom Q (80-100 mesh). Column temperature was 180°C , injector temperature 220°C , venting block 265°C , and pyrolysis furnace 840°C . Retention time for the methyl ester of 2,4-D under these conditions was about 2.2 minutes.

Detection limit of the 2,4-D methyl ester was 1.25 ng with 15 ng giving a 50% deflection on the recorder. Detection limit for fish samples: 0.01 mg/kg; for hydrosol: 0.005 mg/kg; and water: 0.001 mg/liter.

Confirmatory analyses of the 2,4-D methyl ester standard, spiked samples, and periodic samples were conducted by electron capture detection on a Beckman GC-4 gas chromatograph.

Acknowledgment

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Nationwide Organochlorine and Mercury Residues in Wings of Adult Mallards and Black Ducks during the 1969-70 Hunting Season

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ABSTRACT

Nationwide monitoring of organochlorine and mercury residues in wings of approximately 5,200 adult mallards and black ducks bagged during the 1969-70 hunting season showed DDE, as in 1965 and 1966, to be the predominant residue. PCB's were next in overall prevalence, followed by mercury, DDT, dieldrin, DDD, and heptachlor epoxide. There was no indication of a decrease in levels from 1966. Residues were generally highest in the Atlantic and Pacific Flyways and lowest in the Central Flyway; PCB's, however, were highest in the Atlantic Flyway and diminished westward across the Nation. Mercury residues were highest in Atlantic Flyway black ducks. Monitoring results were consistent: States with high residues in 1965 and 1966 were again high in 1969. Analyses of individual wings of mallards from California and black ducks from New Jersey and New York revealed DDE residues as high as 41 ppm and certain regional and seasonal differences in levels within States. They also revealed an apparent tendency for DDE residues to be more variable and to attain higher levels in male than in female wings.

Introduction

The first nationwide monitoring of pesticides in waterfowl occurred in early 1966 when the U.S. Department of the Interior Bureau of Sport Fisheries and Wildlife assessed organochlorine residues in wings of approximately 12,000 mallards (*Anas platyrhynchos*) and black ducks (*Anas rubripes*) bagged during the 1965-66 hunting season. Monitoring was repeated a year later with a similar number of wings from the 1966-67 hunting season, and the data from the two years were combined to provide base readings for measuring trends in future residue levels (1). Monitoring was thereafter scheduled

at 3-year intervals to serve this purpose. The undertaking is a segment of the National Pesticide Monitoring Program; the Bureau's current commitment in that program is described by Dustman et al. (2).

Wings of approximately 5,200 adult mallards and black ducks bagged during the 1969-70 hunting season have been monitored. Findings of that survey are presented in the following report, which includes: (a) average wing levels of DDE, DDT, DDD, dieldrin, PCB's, and mercury among birds bagged in each State; (b) a comparison of 1969-70 levels of DDE and dieldrin with the earlier 2-year averages (DDT and DDD comparisons are biased by earlier PCB interference); and (c) a special study of individual wings from California, New Jersey, and New York to compare residues among birds bagged in different periods and regions within each State.

The wings used in monitoring are a by-product of an established nationwide survey in which, each fall, selected waterfowl hunters mail the Bureau tens of thousands of duck wings for biological examination. An appraisal of their suitability as a medium for monitoring was presented earlier (1,3).

Methods

NATIONWIDE MONITORING

Wings from the 1969-70 waterfowl season were mailed by hunters to one of four regional collection points and held in frozen storage for biological examination in early 1970. Hunters reported the date, State, and county of kill for each wing, and biologists determined the species, sex, and maturity of the bird. Wings of adult mallards from most States, and of adult black ducks from eastern States, were retained for monitoring following biological examination.

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For practicality, monitoring in 1970 was restricted to adult wings; of those chemicals consistently detected in the earlier monitoring (DDE, DDT, DDD, and dieldrin), only DDE proved to have higher residues in adult wings than in immature wings. DDE levels in adults are of particular concern because the chemical has been shown experimentally to cause eggshell thinning in both mallards (4, 5) and black ducks (6).

As in earlier monitoring, the wings from each State were sorted systematically into pools of 25 each, and a random sample of pools roughly proportional in number to the State's mallard or black duck harvest was then selected for chemical analysis. Pools not selected were discarded except for certain mallard wings from California and black duck wings from New York and New Jersey which were retained for individual analyses (see *Findings*, this paper). Each pool or individual wing was enclosed with an individually numbered tag in a plastic bag, packaged in dry ice, and shipped for chemical analysis to WARF Institute, Inc., Madison, Wisconsin. Wing material was identified only by a code number during chemical analysis.

INDIVIDUAL WINGS

In earlier monitoring, variation in pool levels of DDE within certain States with the highest averages was sufficient to indicate that some birds carried residues considerably higher than the respective average. To investigate this likelihood, selected mallard wings from California and black duck wings from New Jersey and New York were analyzed individually to compare residues among birds bagged in different regions of the State and/or during different periods of the hunting season. Regions and periods were chosen, hopefully, to maximize any contrasts in levels. It was hypothesized that residues for these States would tend to be higher in birds taken early in the season when, prior to full-scale migration, the population comprised a higher proportion of local birds.

In California, wings were collected from three regions: a northeast region comprising Lassen, Siskiyou, and Modoc Counties, a second region comprising the counties of the Sacramento Valley, and a third region comprising counties of the San Joaquin Valley. These areas were sampled October 18–November 15, 1969, and January 1–10, 1970. The northeast region, devoted largely to ranching, was expected to yield birds with residues lower than those in birds from the two Valley regions of intensive agriculture to the south.

Residues in black ducks from New Jersey and New York were investigated conjunctively with samples of 28 and 34 wings. In New Jersey, which was not regionalized, wing residues in birds from coastal counties bagged October 13–26 were compared with those bagged December 13–27. New York was regionalized into Long

Island and upstate New York. To investigate whether residues were higher in birds from coastal New Jersey and Long Island than in birds from upstate New York, the latter were sampled October 13–26 for simultaneous comparison with New Jersey, and November 17–30 for comparison with birds bagged concurrently on Long Island.

CHEMICAL METHODOLOGY

Preparatory to analysis, wings were trimmed by removing the distal joint and most of the feathers. The remaining portions were chopped and blended into 25-wing homogenates with a Hobart food chopper. A 40-g aliquot of homogenate was removed for pesticide and PCB analysis, and a separate 10-g aliquot was taken for mercury analysis. The 40-g aliquot was dried at 40°C to constant weight, ground with anhydrous sodium sulfate, and extracted in a Soxhlet for 8 hours with 300 ml of a mixture (70:170) of ethyl ether:petroleum ether. The extract was eluted through a standardized Florisil column with 250 ml of a mixture (3:1) of hexane:benzene, partitioned into acetone:nitrile, and passed a second time through a Florisil column. The eluate was concentrated and made to volume. Half the Florisil eluate was used to measure organochlorine pesticides, and the second half was reserved for PCB analysis. Lipids were measured by drying a 25-ml aliquot of the Soxhlet extract.

Organochlorine pesticides were quantified as follows: Using thin-layer chromatography (TLC), samples were plated on glass-backed Chrom AR 7GF 20 x 20 pre-poured plates. The pesticides and PCB's were developed and separated into four zones on each plate as described by Mulhern (7), except that hexane was used as the developing solvent instead of hexane:ethyl ether. Each zone was analyzed on a 3% OV-17 column, with confirmation on 5% DC-200 and 3% XE-60. Operating specifications are shown in Table 1.

TABLE 1.—Chromatographic operating specifications using electron capture detection

	COLUMNS: GLASS, 4-FT-BY-4-MM INSIDE DIAMETER		
	3% OV-17 Gas Chrom Q 100/120 190°C 3.5(dieldrin)	5% DC-200 Gas Chrom Q 80/100 190°C 6-8(p,p'-DDT)	3% XE-60 Gas Chrom Q 60/80 170°C 10-12(p,p'-DDT)

PCB determinations were derived using semiquantitative thin-layer methods (8), with the modification that 0.1 g instead of 1.0 g AgNO₃ was used in making the plates. All PCB samples were read by comparison of total area with an Aroclor 1254 standard.

Total mercury was determined by cold vapor atomic absorption. The 10-g aliquot was digested by refluxing

with a mixture of sulfuric and nitric acids (9). A mixture of hydroxylamine, stannous chloride, and sulfuric acid was added to the digest to reduce the mercury (II) ions to mercury metal. Samples were aerated (3 liters/min) and passed through the absorption cell.

All residues have been expressed on a wet-weight basis. Limits of sensitivity were 0.02 ppm for organochlorine pesticides, 0.03 ppm for total PCB's, and 0.05 ppm for mercury. Conversion of residue levels to ppm dry weight or ppm lipid weight may be approximated by dividing a given wet-weight value by 0.57 or by 0.13, the respective mean proportions of dry or lipid material in each sample. Precise measurements of dry and lipid proportions for specific samples are available upon request.

The recovery efficiency of the analytical procedures was evaluated by analyzing nine 40-g aliquots of homogenized wing material known to contain only trace quantities of residues. Three of the aliquots remained untreated, three were identically spiked with low levels of five chemicals, and three were spiked with high levels. Spiking included 0.5 ppm or 1.0 ppm *p,p'*-DDT, 0.1 ppm or 0.2 ppm *p,p'*-DDD, 3.0 ppm or 5.0 ppm *p,p'*-DDE, 0.06 ppm or 0.2 ppm dieldrin, and 0.5 ppm or 5.0 ppm PCB as Aroclor 1254. DDT was recovered at an estimated rate of 86%, DDD at 81%, DDE at 96%, dieldrin at 88%, and PCB at 123%. Recovery efficiency for mercury was 97%; the median difference among duplicate analyses of 15 pools was 0.01 ppm mercury, and the coefficient of variation was 22.7%. None of the reported residue data have been adjusted for rates of recovery.

Findings

NATIONWIDE MONITORING

Average residue levels (ppm wet weight) of DDE, DDT, DDD, dieldrin, PCB, and mercury in wings of adult mallards or black ducks in late 1969–early 1970 are listed in Table 2 by State where collected. The table also lists the 2-year averages (1965–66) for DDE and dieldrin, as well as the sample size, range, and standard error associated with each average. States are listed in north to south order within each of the waterfowl flyways (Atlantic, Mississippi, Central, and Pacific) to facilitate geographic comparisons. Table 3 lists the flyway averages for the same chemicals and periods, the averages having been derived by weighting each of the State averages by the estimated State bag of the species.

DDE again proved to be the predominant organochlorine residue in mallard and black duck wings. It was present in measurable amounts in every pool, ranging in concentrations from a low of 0.06 ppm in a mallard pool from Nebraska to a high of 5.27 ppm in a black duck pool from New Jersey. State DDE

averages ranged from 0.09 ppm in South Dakota mallards to 3.42 ppm in New Jersey black ducks.

There was no indication that DDE levels were diminishing during the 3-year period from 1966 to 1969. Residues tended to remain stable in the Pacific and Central Flyways; an apparent increase in the latter was due largely to an unusually high Texas reading. DDE levels tended to have increased in mallards from the Mississippi and Atlantic Flyways; averages were higher in 18 of 21 States. Changes in DDE levels in black ducks were mixed, although there was a tendency for levels to be higher in 1969.

Geographically, State and regional contrasts in DDE levels seen in earlier wing monitoring were again prevalent. Levels in black ducks from the contiguous Atlantic Flyway States extending from New Hampshire to Delaware were among the highest in the survey, although increases in birds from Virginia, North Carolina, and South Carolina resulted in levels nearly as high as those to the north. The highest average DDE residues in the nation occurred in birds from New Jersey, where wings of black ducks averaged 3.42 ppm and mallards 2.62 ppm. Residues were again high in mallards from Alabama (1.75 ppm), California (1.52 ppm), Utah (1.29 ppm), Pennsylvania (1.48 ppm), and New York (0.99 ppm). Mallard wings from certain of the Central Flyway States (Montana, Wyoming, South Dakota, Nebraska, Kansas, and Oklahoma) again carried the lowest DDE residues, averaging from 0.09 ppm in South Dakota to 0.15 ppm in Oklahoma.

DDT residues averaged from approximately one-fifth to one-tenth as high as those of DDE, the highest DDT averages occurring in Utah mallards (0.28 ppm) and New Jersey black ducks (0.27 ppm). Residues of DDD seldom exceeded trace levels. DDT and DDD residues should not be compared with those from earlier wing monitoring, because interference from certain PCB isomers undoubtedly inflated the earlier readings.

Dieldrin levels in mallard wings showed little change from 1965 and 1966 monitoring. The apparent increase in black duck residues in the Atlantic Flyway could not be substantiated statistically because of variability in the data. The highest dieldrin levels were again found in wings from the Atlantic Flyway; the highest averages were in black ducks from Maine (0.44 ppm), Massachusetts (0.18 ppm), Connecticut (0.16 ppm), Rhode Island (0.26 ppm), and Pennsylvania (0.16 ppm). Dieldrin averaged 0.14 ppm in mallards from Pennsylvania, the only State of the above five from which mallards were sampled. Dieldrin averaged approximately 0.18 ppm among black ducks for the three-State area of Virginia and the Carolinas, although residue levels among pools varied more than in the northern group of States. In the remaining flyways, State dieldrin averages exceeded 0.05 ppm only in Ohio, Alabama, Arkansas,

Texas, and Utah. All States except Ohio had been noted for prevalence of dieldrin in previous wing monitoring.

Dieldrin and DDT levels were comparable in black ducks; whereas in mallards, dieldrin residues were nationwide somewhat less than those of DDT. With few exceptions, dieldrin residues exceeded those of DDD.

Heptachlor epoxide was detected in most pools and in every State except Vermont. Few pools contained levels exceeding 0.02 ppm; therefore the chemical was excluded from the tables. No other organochlorine residues of pesticidal origin were reported.

PCB levels exhibited pronounced geographic differences, being highest in the Atlantic Flyway and generally diminishing westward. Residues were highest from New Hampshire southward through Delaware. The highest average PCB levels in the entire survey were found in black ducks from Connecticut (3.88 ppm) and Massachusetts (2.12 ppm). State averages exceeded 0.50 ppm throughout the Atlantic Flyway except in black ducks from Maryland (0.39 ppm). PCB levels in the Mississippi Flyway were highest in birds from States bordering the Great Lakes, diminishing southward in all States except Tennessee. Levels were comparably lower in the Central and Pacific Flyways, averaging overall about 0.15 ppm. The relatively high Nevada average (0.73 ppm) needs further investigation, and the low California average (0.04 ppm) may reflect the fact that there is little heavy industry in that part of the State most frequented by mallards.

Mercury residues of at least 0.05 ppm were detected in all pools of wings. However, levels exceeded 0.50 ppm in only three pools, one each from New York (black ducks, 0.80 ppm), Wisconsin (1.0 ppm), and Nevada (0.55 ppm). Mercury levels in mallards were comparable in all flyways, averaging about 0.10 ppm. Black ducks from the Atlantic Flyway averaged nearly double this level of mercury; averages were slightly higher from Delaware northward.

INDIVIDUAL WINGS, CALIFORNIA

In the following comparisons of residues in wings of individual mallards from California, sexes have been segregated (Table 4). Residues, especially DDE, in a given group of wings tended to be more variable and exhibit higher levels in male than in female wings, although females might have the higher median level. Because underlying statistical distributions were not well understood, all tests of significance have been based on nonparametric methods (10, 11).

In California, as hypothesized, DDE residues in certain mallard wings greatly exceeded the State mean of 1.52 ppm (Table 4). There were also demonstrable differences in average DDE levels between certain seasonal and regional groups of wings. Levels were highest in the

sample of 11 Sacramento Valley males taken during the early period, when the highest proportion of local birds was expected to be in the population; levels ranged from 0.49 ppm to 41.72 ppm, exceeding 3.40 ppm in 7 of 11 wings. To the south, residues in all six early-period males from the San Joaquin Valley exceeded 0.30 ppm, and one wing contained 20.10 ppm DDE. However, in the five early-period males from the northeast counties where ranching predominates, DDE did not exceed 0.30 ppm except in one wing (6.60 ppm). The difference between the average level for Sacramento Valley males and that for males from the northeast counties was statistically significant ($P = 0.05$), and the difference between San Joaquin County males and those from the northeast approached significance ($P < 0.10$). A significant difference could not be shown between the two Valley regions.

In parallel early-period comparisons of DDE residues in female wings, the average level for the San Joaquin Valley was significantly higher ($P < 0.01$) than that for the northeast counties. Comparisons involving Sacramento Valley females were restricted by a shortage of wings.

Regional comparisons of late-period DDE levels necessarily excluded the northeast region where wings were unavailable. The tendency for levels in late-period males to be higher in the San Joaquin Valley (range 0.32-30.65 ppm) than in the Sacramento Valley (0.18-7.13 ppm) was not statistically significant; there were not enough female wings for meaningful comparison.

Seasonal comparisons could be made only within the two Valley regions since late-period wings from the northeast counties were unavailable. In males from the Sacramento Valley, average DDE levels during the early period were significantly higher ($P < 0.01$) than during the late period; levels in 7 of 12 wings from the late period were below the lowest level encountered among the 11 early-period wings. No similar difference was demonstrable in male wings from the San Joaquin Valley, perhaps because relatively fewer migrants winter in the San Joaquin Valley than in the Sacramento Valley. Female wings were too few in number for meaningful comparisons of seasonal differences within either Valley region.

DDE residues in female wings did not exhibit the extreme levels detected in males; differences were apparently due to more than the fact that the sample comprised 39 males compared to 21 females. DDE exceeded 3.00 ppm in only 2 of 21 female wings (4.67 and 3.79 ppm); however, residues exceeded 3.00 ppm in 13 of 39 male wings, the 4 highest of which were 41.72, 30.65, 20.10, and 12.37 ppm. Despite such values, median DDE levels were higher for females in three of the five regional—period groups.

INDIVIDUAL WINGS, NEW JERSEY AND NEW YORK

As with California mallards, sexes were segregated so far as possible in comparing wing residues among individual black ducks from New Jersey and New York (Table 5). Within the set of wings from a given region and period, DDE levels appeared to be higher in male wings than in wings from females or birds of unidentified sex, although differences were not statistically significant, nor so pronounced as in California mallards.

As hypothesized, DDE residues tended to be higher in wings of birds taken in coastal counties than in those from inland counties. The average DDE level in males sampled from coastal New Jersey October 17-26 was significantly higher ($P < 0.01$) than in males from upstate New York during the same period: levels in six of nine New Jersey wings ranged from 3.04 to 13.79 ppm, whereas the highest level in eight New York wings was 2.20 ppm DDE. Similarly, levels in four males from Long Island bagged November 17-30 exceeded those in four males from upstate New York taken during the same period ($P < 0.02$). The same comparisons for female wings showed tendencies similar to those for males, but differences were less pronounced and merely approached statistical significance. Seasonal differences in DDE levels could not be demonstrated either in New Jersey or in upstate New York; Long Island was sampled during only one period. For New Jersey, the average DDE level for males taken December 13-27 was numerically lower than for those bagged October 17-26, but the difference was not statistically significant. Female wings were inadequately represented for comparison.

Discussion

From the data in Table 2, a multitude of statistical comparisons of residue levels is possible both between States and between years within States. In many instances differences clearly are either significant or not significant. Caution is suggested, however, when the distinction is not clear-cut: the individual pool values may facilitate a more rigorous test of significance than is possible using pool means and their standard errors. Caution is also suggested in combining data from adjacent States unless the data have been properly weighted. Individual pool values and weighting factors are available on request.

The precision with which these wing monitoring data can be interpreted has been questioned at times because of the mobility of mallards and black ducks during migration and the subsequent difficulty in associating wing residues with specific geographic locations. The subject has been discussed in previous papers (1, 3), but further elaboration is perhaps warranted.

The foremost objective in monitoring wings of mallards and black ducks is to quantify not only levels, but also

trends in levels of pesticides and pollutants in continental populations of these highly valued waterfowl. Trends may be either in time or space. As stated earlier (1), "Essentially, we are monitoring flyway as well as State populations, the wings from each State being a sample of that part of a flyway population frequenting the State during the hunting season." It was also stated (3), "... the mobility of the species might permit only a general location of the source of contamination, [but] monitoring of localized material (soil, crops, etc.) by other agencies should help identify such concentrations." The same general problem exists in monitoring other motile media, whether air, flowing water, or a migratory species; however, a medium need hardly be fixed in space to warrant monitoring.

Several factors support the hypothesis of positive association between average residue levels in wings from a State and average levels in at least that portion of the State's aquatic environment frequented by waterfowl. These factors include consistency of wing monitoring data, consistency of migration patterns of adult ducks (12), and, in general, rapid dietary assimilation of organochlorine residues (13, 14).

Summary

Nationwide monitoring of organochlorine compounds and mercury in wings of adult mallards and black ducks from the 1969-70 hunting season showed that all residues, except those of PCB's, were highest in the two coastal flyways, intermediate in the Mississippi Flyway, and lowest in the Central Flyway. PCB residues followed a similar pattern except in the Pacific Flyway, where residues were generally the lowest in the nation.

DDE was again the predominant residue. Average levels failed to suggest a decrease from 1966 and, in fact, increased in mallards in 18 of 21 States sampled in the Atlantic and Mississippi Flyways. No changes in dieldrin levels could be substantiated statistically, although the average level appeared to increase in the Atlantic Flyway. PCB and mercury were not previously quantified, and DDT and DDD should not be compared with earlier measurements because they undoubtedly include some PCB contamination.

Analyses of individual mallard wings from California and black duck wings from New Jersey and New York for residues of organochlorine pesticides revealed DDE residues as high as 41 ppm in the mallards and 13.8 ppm in black ducks, whereas the highest respective DDE levels among 25-wing pools were 2.70 ppm and 5.27 ppm. Analyses of individual wings from California showed that residues were higher in birds from the intensively cultivated Sacramento and San Joaquin Valleys than from the northeastern ranching counties. In New Jersey and New York, birds from the coastal counties contained significantly higher residues than did those

from upstate New York. In both instances the data suggest a tendency for residues to be more variable and attain higher levels in male than in female wings, although the median level might be higher in the female wings of a given set.

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TABLE 2.—Fall residue¹ levels of DDE, DDT, DDD, dieldrin, PCBs, and mercury in pools of 25 wings of adult mallards or black ducks: 1969 compared with 1965-66

STATE; YEAR	NO. OF POOLS	RESIDUES, PPM (WET WEIGHT)																	
		DDE			DDT			DDD			DIELDRIN			PCB's			MERCURY		
		MEAN	EXTREME VALUES	STD. ERROR	MEAN	EXTREME VALUES	STD. ERROR	MEAN	EXTREME VALUES	STD. ERROR	MEAN	EXTREME VALUES	STD. ERROR	MEAN	EXTREME VALUES	STD. ERROR	MEAN	EXTREME VALUES	STD. ERROR
BLACK DUCKS, ATLANTIC FLYWAY																			
Maine 1969 1965-66	3 4 + 4	0.60 0.48	0.33-0.85 0.32-0.82	0.122 0.064	0.05	0.03-0.07	0.009	(0.01) ²	all t ³	(0.007)	0.44 (0.02)	t-1.72 —	0.426 (0.006)	0.79	0.20-1.64	0.332	0.19	0.16-0.24	0.017
Vt. 1969 1965-66	2 3 + 3	0.66 0.75	0.60-0.71 0.18-2.10	0.055 0.279	0.03	0.03-0.03	0	(0.01)	all t	(0.010)	(0.01) (0.02)	all t —	(0.010) (0.008)	0.83	0.33-1.33	0.500	0.26	0.14-0.39	0.125
N.H. 1969 1965-66	2 3 + 3	0.87 0.88	0.80-0.93 0.48-2.17	0.065 0.262	0.04	t-0.06	0.025	(0.01)	all t	(0.010)	0.02 0.10	0.02-0.02 n ⁴ -0.55	0 0.092	1.50	1.33-1.67	0.170	0.20	0.20-0.20	0
Mass. 1969 1965-66	4 4 + 4	1.90 1.69	1.11-2.34 1.28-2.67	0.286 0.166	0.09	t-0.19	0.038	0.04	0.02-0.05	0.007	0.18 0.10	0.05-0.49 n-0.38	0.105 0.045	2.12	1.64-3.00	0.303	0.22	0.21-0.22	0.003
Conn. 1969 1965-66	3 3 + 3	1.06 1.51	0.98-1.22 0.77-2.62	0.078 0.311	0.13	0.09-0.16	0.022	0.02	0.02-0.03	0.003	0.16 0.06	0.08-0.22 n-0.18	0.042 0.029	3.88	2.67-5.00	0.674	0.21	0.19-0.24	0.017
R.I. 1969 1965-66	3 3 + 3	1.36 0.94	1.27-1.45 0.56-1.50	0.052 0.156	0.18	0.15-0.24	0.029	0.03	0.02-0.03	0.003	0.26 0.21	0.23-0.29 n ⁴ -0.43	0.018 0.061	1.58	1.00-1.96	0.295	0.19	0.14-0.22	0.024
N.Y. 1969 1965-66	4 4 + 4	1.19 1.24	0.90-1.69 0.78-1.58	0.174 0.092	0.13	0.02-0.23	0.043	0.02	0.02-0.03	0.003	0.03 0.05	0.02-0.04 n-0.15	0.004 0.021	1.64	0.25-3.00	0.578	0.33	0.18-0.80	0.157
Pa. 1969 1965-66	3 3 + 3	0.78 1.14	0.70-0.91 0.33-3.60	0.067 0.511	0.06	0.04-0.07	0.009	(0.01) ²	all t ³	(0.007)	0.16 (0.03)	0.03-0.30 n-0.10	0.078 (0.016)	1.00	0.50-1.67	0.348	0.16	0.12-0.19	0.020
N.J. 1969 1965-66	5 5 + 5	3.42 2.10	2.31-5.27 1.32-3.45	0.520 0.228	0.27	0.18-0.35	0.036	0.09	0.06-0.12	0.010	0.06 (0.03)	0.02-0.12 n-0.08	0.018 (0.010)	0.92	0.13-1.66	0.295	0.18	0.15-0.23	0.014
Del. 1969 1965-66	2 3 + 3	1.54 0.88	1.52-1.55 0.50-1.50	0.015 0.171	0.12	0.11-0.12	0.005	0.04	0.03-0.04	0.005	0.03 0.05	0.03-0.03 n-0.19	0 0.032	1.06	0.62-1.50	0.440	0.20	0.15-0.25	0.050
Md. 1969 1965-66	3 3 + 3	0.55 0.33	0.47-0.63 0.13-0.51	0.046 0.056	0.06	0.05-0.08	0.009	(0.01) ²	all t ³	(0.007)	0.03 (0.03)	0.02-0.03 n ⁴ -0.06	0.003 (0.010)	0.39	0.10-0.83	0.223	0.11	0.10-0.13	0.009
Va. 1969 1965-66	3 3 + 3	0.89 0.40	0.64-1.18 0.20-0.72	0.157 0.077	0.14	0.04-0.30	0.081	0.02	t-0.03	0.006	0.13 0.11	t-0.32 n-0.43	0.096 0.071	1.17	0.50-2.00	0.441	0.15	0.13-0.17	0.012
N.C. 1969 1965-66	2 1 + 2	0.80 0.48	0.74-0.86 0.31-0.66	0.060 0.101	0.08	0.06-0.09	0.015	0.02	0.02-0.03	0.005	0.30 (0.02)	0.03-0.57 —	0.270 (0.017)	0.66	0.49-0.83	0.170	0.15	0.14-0.16	0.010
S.C. 1969 1965-66	2 2 + 2	1.03 0.30	0.76-1.30 0.18-0.53	0.270 0.081	0.06	0.05-0.07	0.010	0.03	0.02-0.04	0.010	0.10 (0.03)	0.06-0.15 n-0.07	0.045 (0.017)	1.25	0.50-2.00	0.750	0.18	0.18-0.19	0.005
MALLARDS, ATLANTIC FLYWAY																			
N.Y. 1969 1965-66	3 3 + 3	0.99 1.24	0.86-1.11 0.52-2.15	0.072 0.237	0.10	0.07-0.14	0.020	0.02	0.02-0.03	0.003	0.02 T ⁵	t ³ -0.03 n ⁴ -0.11	0.007 —	1.33	0.33-2.00	0.511	0.11	0.09-0.12	0.009
Pa. 1969 1965-66	3 3 + 3	1.48 0.78	0.92-2.61 0.37-1.50	0.563 0.189	0.05	0.04-0.07	0.010	(0.01) ²	all t	(0.007)	0.14 0.05	0.03-0.35 n-0.16	0.105 0.027	1.00	0.33-1.67	0.387	0.08	0.06-0.10	0.012

TABLE 2.—Fall residue^a levels of DDE, DDT, DDD, dieldrin, PCBs, and mercury in pools of 25 wings of adult mallards or black ducks: 1969 compared with 1965-66

STATE; YEAR	No. of POOLS	RESIDUES, IN PPM (WET WEIGHT)																	
		DDE			DDT		DDD		DIELDRIN		PCB'S		MERCURY						
		MEAN	EXTREME VALUES	STD. ERROR	MEAN	EXTREME VALUES	STD. ERROR	MEAN	EXTREME VALUES	STD. ERROR	MEAN	EXTREME VALUES	STD. ERROR	MEAN	EXTREME VALUES	STD. ERROR			
MALLARDS, ATLANTIC FLYWAY—Continued																			
N.J. 1969	3	2.62	2.38-2.86	0.240	0.22	0.17-0.26	0.045	0.05	0.05-0.05	0	0.07	0.05-0.09	0.020	5.94	0.18-1.17	5.76	0.11	0.10-0.12	0.010
1965-66	3 + 3	1.08	0.40-2.60	0.383							T	n-0.06	—						
Md. 1969	2	0.55	0.38-0.77	0.116	0.03	t-0.05	0.012	(0.01)	all t	(0.010)	(0.01)	t-0.02	(0.007)	0.62	0.20-1.33	0.357	0.08	0.08-0.08	0
1965-66	3 + 2	0.44	0.20-0.84	0.118							0.05	n-0.17	0.034						
Va. 1969	2	0.45	0.41-0.48	0.035	0.22	0.05-0.40	0.175	0.03	0.02-0.03	0.005	0.04	t-0.07	0.030	0.56	0.13-1.00	0.435	0.16	0.15-0.16	0.005
1965-66	3 + 3	0.30	0.19-0.59	0.061							0.06	n-0.11	0.018						
S.C. 1969	3	0.65	0.44-0.94	0.151	0.14	0.09-0.22	0.040	0.02	0.02-0.02	0	0.02	0.02-0.03	0.003	0.52	0.17-0.99	0.244	0.13	0.11-0.15	0.012
1965-66	3 + 3	0.37	0.13-1.00	0.133							0.05	n + 0.16	0.026						
Ga. & Fla. 1969	2	0.82	0.49-1.32	0.255	0.06	0.05-0.06	0.003	(0.01) ²	t ± 0.02	(0.010)	0.02	t-0.02	0.003	0.97	0.25-1.67	0.410	0.12	0.09-0.18	0.029
1965-66	2 + 2	0.53	0.35-0.70	0.079							n	—	—						
MALLARDS, MISSISSIPPI FLYWAY																			
Minn. 1969	5	0.30	0.20-0.42	0.040	0.04	0.02-0.08	0.010	T ^o	all t	—	(0.01)	all t	(0.004)	0.70	0.16-1.33	0.214	0.08	0.06-0.10	0.007
1965-66	7 + 6	0.24	0.08-0.52	0.039							n	—	—						
Wisc. 1969	5	0.34	0.19-0.48	0.055	0.03	0.02-0.05	0.005	T	all t	—	(0.01)	all t	(0.004)	0.58	0.17-1.00	0.179	0.26	0.05-1.00	0.184
1965-66	3 + 5	0.28	0.08-0.81	0.096							T	n-0.05	—						
Mich. 1969	3	0.45	0.33-0.53	0.061	0.05	0.02-0.08	0.018	T ^o	t ± 0.02	—	0.03	0.02-0.05	0.010	0.62	t-1.00	0.302	0.11	0.08-0.14	0.017
1965-66	2 + 3	0.22	0.10-0.48	0.067							n ⁴	—	—						
Iowa 1969	4	0.33	0.16-0.54	0.094	0.05	t-0.09	0.019	T	all t	—	0.03	0.02-0.05	0.006	0.13	0.07-0.25	0.041	0.08	0.06-0.10	0.009
1965-66	5 + 5	0.22	0.06-0.92	0.080							n	—	—						
Ill. 1969	6	0.73	0.18-2.69	0.395	0.12	0.02-0.54	0.085	0.05	t-0.26	0.043	0.04	0.02-0.07	0.008	0.36	0.13-1.00	0.131	0.06	0.05-0.08	0.005
1965-66	7 + 6	0.09	n-0.27	0.020							T	n-0.06	—						
Ind. 1969	1	0.19	—	—	0.02	—	—	T	—	—	0.03	—	—	1.00	—	—	0.06	—	—
1965-66	4 + 3	0.17	0.06-0.29	0.034							T	n-0.05	—						
Ohio 1969	3	0.38	0.24-0.54	0.087	0.18	0.05-0.30	0.073	T	t-0.02	—	0.09	0.04-0.15	0.033	0.66	0.03-1.66	0.504	0.10	0.08-0.12	0.012
1965-66	3 + 3	0.24	0.15-0.31	0.028							n	—	—						
Mo. 1969	5	0.20	0.13-0.30	0.029	0.02	t ± 0.04	0.007	T ^o	all t	—	0.05	0.04-0.06	0.004	0.21	0.10-0.33	0.052	0.07	0.05-0.11	0.012
1965-66	6 + 5	0.13	n-0.72	0.065							n ⁴	—	—						
Ky. 1969	1	0.33	—	—	0.04	—	—	T	—	—	0.03	—	—	0.33	—	—	0.09	—	—
1965-66	3 + 3	0.30	0.13-0.62	0.080							n	—	—						
Ark. 1969	6	0.25	0.21-0.28	0.013	0.12	0.04-0.16	0.019	0.03	t-0.04	0.004	0.06	0.02-0.11	0.013	0.16	t-0.67	0.103	0.08	0.06-0.10	0.007
1965-66	7 + 6	0.20	0.08-0.31	0.021							0.08	n-0.34	0.028						
Tenn. 1969	4	0.26	0.20-0.34	0.029	0.06	0.02-0.11	0.023	T	all t	—	0.04	0.02-0.04	0.005	0.71	0.25-1.66	0.333	0.08	0.07-0.12	0.012
1965-66	5 + 4	0.33	0.11-1.10	0.099							0.06	n-0.39	0.043						

L.a. 1969 1965-66	5 5 + 6	0.44 0.18	0.27-0.74 0.05-0.26	0.081 0.018	0.11	0.07-0.19	0.026	0.02	t-0.03	0.003	0.05 T	0.04-0.07 n-0.05	0.007 —	0.23	0.07-0.50	0.074	0.08	0.06-0.09	0.005
Miss. 1969	2 3 + 3	0.52 0.51	0.36-0.68 0.21-0.96	0.160 0.137	0.14	0.05-0.22	0.009	0.04	0.04-0.05	0.005	T ⁵	0.04-0.05 n-0.05	0.005 —	0.10	t ² -0.17	0.075	0.08	0.07-0.08	0.005
Ala. 1969	1 2 + 3	1.75 2.17	— 0.54-5.31	— 0.922	0.09	—	—	2.07	—	—	0.05 0.09	— n-0.14	0.035 —	0.42	—	—	0.06	—	—

MALLARDS, CENTRAL FLYWAY

Mont. (east) 1969	4 8 + 4	0.11 0.09	0.08-0.17 0.05-0.16	0.020 0.010	0.03	t-0.08	0.017	0.02	t-0.03	0.008	0.04 T	t-0.05 n-0.16	0.010 —	0.18	0.13-0.25	0.025	0.07	0.05-0.09	0.008
N. Dak. 1969	6 8 + 8	0.25 0.12	0.14-0.44 0.05-0.43	0.050 0.022	0.03	t-0.06	0.008	T	all t	—	(0.01) ² n	all t —	(0.003) —	0.13	t-0.50	0.076	0.08	0.06-0.11	0.008
S. Dak. 1969	6 7 + 8	0.09 0.11	0.07-0.14 n-0.20	0.011 0.014	(0.01)	t-0.02	(0.003)	T	n-t	—	(0.01) n	all t —	(0.003) —	0.13	t-0.25	0.034	0.06	0.05-0.09	0.006
Wyo. (east) 1969	3 1 + 3	0.13 0.16	0.08-0.19 0.05-0.45	0.032 0.097	0.02	t ² -0.03	0.009	T ⁵	all t	—	0.02 n ⁴	t-0.02 —	0.003 —	0.30	0.25-0.33	0.027	0.08	0.07-0.09	0.006
Neb. 1969	7 6 + 7	0.13 0.10	0.06-0.19 0.05-0.17	0.017 0.009	(0.01) ²	t-0.02	(0.003)	T	all t	—	(0.01) n	t-0.02 —	(0.004) —	0.06	t-0.13	0.015	0.09	0.07-0.13	0.010
Colo. (east) 1969	4 10 + 4	0.46 0.30	0.13-1.25 0.17-0.85	0.266 0.048	0.06	0.02-0.09	0.015	T	t-0.02	—	0.02 n	t-0.03 —	0.006 —	0.43	0.07-1.33	0.301	0.08	0.07-0.10	0.007
Kans. 1969	6 7 + 6	0.11 0.08	0.08-0.15 n-0.12	0.011 0.009	0.02	t-0.06	0.008	T	t-0.02	—	0.02 n	t-0.03 —	0.005 —	0.15	0.03-0.50	0.072	0.06	t-0.09	0.010
N.M. (east) 1969	3 3 + 3	0.37 0.74	0.26-0.57 0.22-2.44	0.099 0.349	0.03	0.02-0.05	0.009	T	all t	—	0.02 T	t-0.02 n-0.06	0.003 —	0.18	0.13-0.25	0.037	0.09	0.06-0.10	0.013
Okl. 1969	4 5 + 4	0.15 0.11	0.13-0.18 0.07-0.20	0.012 0.016	0.02	0.02-0.02	0	T ⁵	t ² -0.02	—	0.03 n ⁴	t-0.08 —	0.019 —	0.12	t-0.23	0.058	0.08	0.05-0.11	0.013
Tex. 1969	6 6 + 9	0.98 0.35	0.13-4.38 0.12-1.14	0.683 0.072	0.09	t-0.38	0.059	T	t-0.02	—	0.07 0.08	t-0.26 n-0.53	0.039 0.037	0.20	t-0.67	0.100	0.06	0.05-0.07	0.003

MALLARDS, PACIFIC FLYWAY

Wash. 1969	8 13 + 11	0.36 0.47	0.19-0.48 0.06-2.75	0.037 0.125	0.04	0.03-0.05	0.003	T	t-0.02	—	(0.01) ² T	t-0.02 n-0.10	(0.004) —	0.14	t-0.33	0.039	0.09	0.05-0.18	0.014
Oreg. 1969	7 7 + 7	0.48 0.36	0.21-1.03 0.12-0.59	0.122 0.038	0.09	0.03-0.29	0.037	T	t-0.03	—	0.03 T	t-0.12 n-0.09	0.017 —	0.10	t-0.20	0.020	0.09	0.06-0.10	0.006
Idaho 1969	8 9 + 9	0.48 0.51	0.23-0.79 0.06-2.77	0.069 0.140	0.08	0.05-0.22	0.021	T ⁵	t ² -0.02	—	0.02 T	t-0.05 n ⁴ -0.09	0.006 —	0.14	0.10-0.32	0.028	0.10	0.08-0.15	0.009
Mont. (west) 1969	4 4 + 4	0.14 0.17	0.07-0.21 0.08-0.34	0.029 0.031	0.03	t-0.05	0.009	T	all t	—	(0.01) ² T	all t n-0.06	(0.005) —	0.15	0.03-0.27	0.050	0.10	0.10-0.11	0.003
Wyo. (west) 1969	2 2 + 3	0.12 0.07	0.09-0.14 n-0.10	0.025 0.015	0.02	t-0.03	0.010	T	all t	—	0.02 n	t-0.03 —	0.010 —	0.12	0.10-0.13	0.015	0.12	0.10-0.13	0.015
Calif. 1969	8 11 + 11	1.52 1.45	0.99-2.70 0.02-3.50	0.190 0.174	0.19	0.09-0.37	0.035	0.04	t-0.22	0.026	0.02 T	t-0.10 n-0.08	0.012 —	0.04	t-0.10	0.013	0.08	0.05-0.11	0.007

TABLE 3.—Mean residues¹ of DDE, DDT, DDD, and dieldrin, by waterfowl flyways, in pools of 25 wings of adult mallards or black ducks in 1969 and in 1965-66; and residues of PCB's and mercury in 1969

SPECIES	FLYWAY	YEAR	No. OF POOLS	RESIDUES, PPM (WET WEIGHT)											
				DDE		DDT		DDD		DIELDRIN		PCB's		MERCURY	
				MEAN	STD. ERROR	MEAN	STD. ERROR	MEAN	STD. ERROR	MEAN	STD. ERROR	MEAN	STD. ERROR	MEAN	STD. ERROR
Black Duck	Atlantic	1969	41	1.42	0.103	0.12	0.011	0.03	0.019	0.14	0.057	1.20	0.124	0.19	0.022
		1965-66	89	1.23	0.078					0.05	0.008	n.a. ²		n.a.	
Mallard	Atlantic	1969	18	1.05	0.139	0.09	0.014	0.02	0.002	0.05	0.025	1.29	0.457	0.10	0.004
		1965-66	39	0.72	0.078					T ³	—	n.a.		n.a.	
Mallard	Mississippi	1969	51	0.40	0.058	0.08	0.012	(0.05) ⁴	—	0.04	0.003	0.35	0.044	0.09	0.014
		1965-66	123	0.25	0.024					T	—	n.a.		n.a.	
Mallard	Central	1969	49	0.30	0.098	0.03	0.009	T	—	0.02	0.006	0.17	0.039	0.08	0.003
		1965-66	117	0.17	0.017					T	—	n.a.		n.a.	
Mallard	Pacific	1969	51	0.71	0.054	0.11	0.012	T	—	0.02	0.005	0.13	0.014	0.10	0.004
		1965-66	117	0.70	0.063					T	—	n.a.		n.a.	

¹ See table 2 for limits of quantification and caution regarding DDT or DDD comparisons.

² n.a. = no analysis.

³ T = mean residue level probably lower than limit of quantification.

⁴ Parenthesized approximation involving trace residues.

TABLE 4.—Pesticide residues in wings of individual adult mallards: California, 1969-70

OCTOBER 18-NOVEMBER 15, 1969							JANUARY 1-10, 1970						
COUNTY	DATE	SEX	RESIDUE, PPM (WET WEIGHT)				COUNTY	DATE	SEX	RESIDUE, PPM (WET WEIGHT)			
			DDE	DDD	DDT	DIELDRIN				DDE	DDD	DDT	DIELDRIN
NORTHEASTERN COUNTIES													
Modoc	Oct 18	M	0.13	t ¹	t	t	No January wings available						
Siskiyou	Oct 18	M	0.18	t	t	t							
Lassen	Oct 18	M	0.20	t	0.02	t							
Siskiyou	Oct 19	M	0.29	t	0.02	t							
Siskiyou	Oct 20	M	6.60	t	0.42	t							
Modoc	Oct 18	F	0.26	t	t	t							
Modoc	Oct 18	F	0.38	t	0.02	t							
Modoc	Oct 18	F	0.41	t	0.02	t							
Modoc	Oct 18	F	0.63	t	0.03	t							
Siskiyou	Oct 19	F	1.08	t	0.14	t							
	Median	M	0.20	t	0.02	t							
		F	0.41	t	0.02	t							
SACRAMENTO VALLEY COUNTIES													
Sutter	Oct 25	M	0.49	t	0.10	0.02	Glenn	Jan 7	M	0.18	t	0.02	t
Yolo	Oct 18	M	0.62	t	0.09	t	Yuba	Jan 3	M	0.25	t	0.03	t
Colusa	Oct 25	M	0.82	0.04	0.21	0.03	Colusa	Jan 8	M	0.28	t	0.02	t
Butte	Oct 19	M	1.21	0.02	0.20	t	Glenn	Jan 9	M	0.31	0.02	0.04	t
Sutter	Oct 25	M	3.47	0.04	0.18	0.02	Butte	Jan 3	M	0.44	t	0.12	0.03
Butte	Oct 19	M	4.86	t	0.22	t	Butte	Jan 10	M	0.45	0.02	0.08	t
Sutter	Oct 18	M	5.05	0.10	0.26	0.06	Colusa	Jan 4	M	0.48	t	0.05	t
Colusa	Oct 25	M	5.91	0.02	0.69	0.03	Glenn	Jan 7	M	0.70	t	0.06	0.03
Yolo	Oct 18	M	6.67	0.04	0.57	0.02	Colusa	Jan 7	M	1.48	0.04	0.06	t
Colusa	Oct 25	M	12.37	0.02	0.20	t	Yolo	Jan 9	M	1.62	0.02	0.33	t
Glenn	Oct 25	M	41.72	0.11	2.46	0.03	Butte	Jan 9	M	2.66	0.06	0.48	t
Sacramento	Oct 18	F	0.18	t	0.03	t	Colusa	Jan 7	M	7.13	0.05	0.40	0.02
Sutter	Oct 18	F	0.47	t	0.10	0.02	Placer	Jan 7	F	0.94	0.02	0.17	t
Colusa	Oct 25	F	1.45	0.03	0.55	0.03	Colusa	Jan 3	F	3.79	t	0.11	t
Colusa	Oct 25	F	1.73	t	0.14	0.02	Butte	Jan 7	F	4.67	0.41	0.72	0.03
	Median	M	4.86	t	0.22	t		Median	M	0.47	t	0.07	t
		F	0.96	0.02	0.33	0.03			F	3.79	t	0.11	t
SAN JOAQUIN VALLEY COUNTIES													
Fresno	Nov 9	M	0.32	t	0.09	0.03	Contra Costa	Jan 9	M	0.32	t	0.05	t
Fresno	Oct 22	M	0.58	0.02	0.07	0.02	Fresno	Jan 1	M	0.44	t	0.08	t
Fresno	Oct 18	M	0.66	0.02	0.15	t	Merced	Jan 3	M	0.60	t	0.05	t
San Joaquin	Oct 19	M	0.86	t	0.05	t	San Joaquin	Jan 9	M	4.58	t	0.11	t
Fresno	Nov 10	M	4.20	0.05	0.47	t	Fresno	Jan 1	M	30.65	0.51	6.65	0.11
Fresno	Oct 22	M	20.10	t	0.23	t	Merced	Jan 3	F	0.13	t	0.03	t
Merced	Oct 22	F	0.44	t	0.05	t	Merced	Jan 3	F	0.15	t	0.02	t
San Joaquin	Oct 19	F	1.24	0.05	0.97	0.02							
Merced	Oct 18	F	1.26	0.03	0.53	0.02							
Merced	Nov 15	F	1.49	t	0.07	t							
Fresno	Oct 19	F	1.51	0.04	0.39	0.02							
Fresno	Oct 19	F	1.78	0.02	0.17	0.12							
San Joaquin	Nov 8	F	2.02	0.03	0.58	0.02							
	Median	M	0.76	t	0.10	t		Median	M	0.60	t	0.05	t
		F	1.49	t	0.07	t			F	0.14	t	0.02	t

¹ t: residue detected at level below limit of quantification.

TABLE 5.—Pesticide residues in wings of individual adult black ducks: New Jersey and New York, late 1969

OCTOBER 17-26, 1969							DECEMBER 13-27, 1969							
COUNTY	DATE	SEX	RESIDUE, PPM (WET WEIGHT)				COUNTY	DATE	SEX	RESIDUE, PPM (WET WEIGHT)				
			DDE	DDD	DDT	DIELDRIN				DDE	DDD	DDT	DIELDRIN	
NEW JERSEY: COASTAL AND DELAWARE BAY COUNTIES														
Cumberland	Oct 21	M	0.51	0.02	0.08	t ¹	(Closed: November 17-30, 1969)	Atlantic	Dec 22	M	0.36	0.02	0.05	0.02
Atlantic	Oct 25	M	0.86	0.02	0.13	t		Cumberland	Dec 13	M	0.75	0.02	0.09	0.02
Ocean	Oct 20	M	1.88	t	0.05	t		Cumberland	Oct 26	M	0.96	0.02	0.08	0.02
Bergen	Oct 18	M	3.04	0.06	0.26	0.07		Atlantic	Dec 23	M	1.30	0.02	0.14	t
Cumberland	Oct 18	M	3.19	0.02	0.39	t		Hudson	Dec 19	M	2.43	0.14	0.40	0.25
Cape May	Oct 23	M	4.74	0.15	0.48	0.02		Atlantic	Dec 20	M	3.44	0.03	0.36	0.03
Atlantic	Oct 23	M	6.00	0.04	0.46	0.03		Ocean	Dec 19	M	5.20	0.15	0.59	0.02
Atlantic	Oct 24	M	8.08	0.03	0.54	t								
Cape May	Oct 22	M	13.79	t	0.14	t								
Bergen	Oct 23	F	0.41	t	0.02	t		Bergen	Dec 23	F	0.41	0.04	0.10	0.08
Bergen	Oct 23	F	3.96	lost	0.70	0.82		Bergen	Dec 27	F	0.61	0.27	0.12	0.30
Ocean	Oct 23	Unk	0.17	t	0.03	t		Cumberland	Dec 27	Unk	0.74	0.02	0.10	0.03
Cape May	Oct 25	Unk	1.83	0.03	0.04	t		Cumberland	Dec 24	Unk	0.98	t	0.07	t
								Cumberland	Dec 27	Unk	1.28	0.02	0.10	0.03
								Cumberland	Dec 27	Unk	1.65	0.08	0.43	0.06
								Cape May	Dec 13	Unk	2.97	0.02	0.22	0.08
								Essex	Dec 26	Unk	4.11	0.02	0.23	0.04
	Median	M	3.19	0.02	0.39	t		Median	M	1.30	0.02	0.14	0.02	
		F	2.19	—	0.36	0.41			F	0.51	0.16	0.11	0.19	
OCTOBER 17-26, 1969							NOVEMBER 17-30, 1969							
COUNTY	DATE	SEX	RESIDUE, PPM (WET WEIGHT)				COUNTY	DATE	SEX	RESIDUE, PPM (WET WEIGHT)				
			DDE	DDD	DDT	DIELDRIN				DDE	DDD	DDT	DIELDRIN	
UPSTATE NEW YORK														
Wayne	Oct 25	M	0.14	t	t	t	Ontario	Nov 24	M	0.30	0.04	0.02	0.02	
Delaware	Oct 18	M	0.15	t	t	t	Ontario	Nov 22	M	0.49	t	0.04	t	
Monroe	Oct 24	M	0.20	t	t	t	Ontario	Nov 22	M	0.82	0.02	0.06	0.05	
Schoharie	Oct 26	M	0.45	t	0.03	t	Niagara	Nov 22	M	1.07	0.06	0.24	0.05	
Dutchess	Oct 18	M	0.75	0.02	0.03	0.02								
Erie	Oct 17	M	1.71	t	0.03	t	Cayuga	Nov 24	F	0.05	t	t	t	
Orange	Oct 23	M	1.77	t	0.05	t	Ontario	Nov 20	F	0.06	t	t	t	
Washington	Oct 21	M	2.20	t	0.02	t	St. Lawrence	Nov 30	F	0.16	t	0.04	0.02	
							Wayne	Nov 23	F	0.16	t	t	t	
Fulton	Oct 21	F	0.17	t	0.02	t	Ontario	Nov 23	F	0.18	t	0.02	t	
Seneca	Oct 23	F	0.18	t	0.02	t	Chautaugua	Nov 30	F	0.73	0.02	0.05	0.05	
Jefferson	Oct 18	F	0.42	t	0.02	0.02	Ontario	Nov 23	F	3.08	t	0.18	0.03	
							Westchester	Nov 17	Unk	0.28	0.02	0.03	0.03	
Jefferson	Oct 20	Unk	0.22	t	t	t		Median	M	0.66	0.03	0.05	0.035	
	Median	M	0.60	t	0.025	t			F	0.16	t	0.02	t	
		F	0.18	t	0.02	t								
NOVEMBER 17-30, 1969														
COUNTY	DATE	SEX	RESIDUE, PPM (WET WEIGHT)				COUNTY	DATE	SEX	RESIDUE, PPM (WET WEIGHT)				
			DDE	DDD	DDT	DIELDRIN				DDE	DDD	DDT	DIELDRIN	
LONG ISLAND														
Nassau	Nov 29	M	1.18	0.02	0.43	0.03								
Suffolk	Nov 24	M	1.48	0.03	0.38	0.02								
Suffolk	Nov 25	M	3.19	0.03	0.58	0.02								
Suffolk	Nov 20	M	3.24	0.08	1.11	0.04								
Nassau	Nov 24	F	0.20	t	0.03	t								
Nassau	Nov 22	F	0.27	t	0.02	t								
Nassau	Nov 22	F	0.43	0.02	0.14	0.03								
Nassau	Nov 25	F	0.80	0.02	0.16	0.02								
Suffolk	Nov 22	Unk	0.46	0.02	0.08	0.06								
Suffolk	Nov 22	Unk	3.87	0.04	0.85	0.02								
	Median	M	2.34	0.03	0.48	0.02								
		F	0.35	0.015	0.085	0.015								
No December wings available														

No December wings available

¹ t: residue detected at level below limit of quantification.

Organochlorine Insecticide Residues in Sediment and Fish Tissues, Ontario, Canada

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ABSTRACT

River and lake sediments and several species of fish were collected from four study areas in the Province of Ontario. These consisted of one agricultural, two mixed agricultural—recreational, and one recreational study area. Large volumes of DDT and dieldrin were used in the first area, small quantities were used in the mixed areas, and insignificant quantities were used in the recreational area. The agricultural and mixed areas were located on deep alluvial soils; the recreational area was located on bare-to-thinly-covered Precambrian rock. Residue levels in sediments were similar in all four areas, but slightly higher in the recreational area. The ratios of DDT to its metabolites, DDE and TDE, were similar in all areas in that the metabolites predominated over the parent DDT. Residues of DDT and dieldrin in fish tissues tended to depend on feeding habits, fat content, and age of the fish. Residues in the sediment (dry weight) were almost the same as residues found in the low-fat benthic invertebrate and plankton feeders.

Residues increased in the low-fat piscivores, were slightly higher in the high-fat feeders, and were highest in the high-fat piscivores. The concentration of DDT and dieldrin in the tissues and extractable fat and the actual quantity accumulated per fish increased as size and weight of any one species increased. The increase in total DDT or dieldrin concentration from lowest to highest tissue residues of all fish species was of the order of 100 to 500 times; the increase in body load was of the order of 10^2 to 10^3 . Higher concentrations in fish tissue were correlated with the higher sediment levels of the Precambrian recreational area. Data demonstrate that the use of even minute quantities of persistent chlorinated hydrocarbons in rocky Precambrian water-

sheds has a profound effect on sediment and biota contaminants. Use on deep rich soils has a similar effect, but not in the same proportion to the quantity used.

Introduction

The persistent organochlorine insecticides have been used for insect control over the past 25 years in the Province of Ontario. In the early years, DDT was registered for a multiplicity of uses in agriculture and forestry. During the 1950's it was used extensively by growers of tobacco, vegetables, and fruit, and was virtually eliminated from use by producers of dairy animals and other livestock. As agricultural and forestry uses became more specific, private and municipal uses became more general; DDT was used in the protection of shade trees and ornamentals and the control of biting flies in urban and recreational areas. Aldrin and dieldrin had special properties for the control of soil-borne insects injurious to crop production. Aldrin was used in urban areas to protect lawns, and dieldrin was used in industry to protect fabrics and timber products.

The persistence of these three insecticides has been reviewed previously (1). A range of 4 to 30 years for DDT and 5 to 25 years for dieldrin has been quoted for 95% disappearance from agricultural soils in the temperate zone. Unfortunately, this persistence is greatly extended because DDT breaks down into two persistent metabolites, TDE and DDE, and dieldrin is derived from aldrin which can persist for 1 to 6 years (1).

Dieldrin and DDT are of very low solubility in water (0.25 to 1.0 ppm and 0.1 to 3.7 ppb, respectively) and are tenaciously held by soil particles; hence movement through soils is insignificant (1). Movement into the

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aquatic environment on soil transported by wind or water is well documented (1, 2). In the aquatic environment DDT is reductively dechlorinated to TDE (3,4), especially by coliform bacteria (3, 5). One study (6) reported that a similar conversion occurs in soils under anaerobic conditions although normal breakdown under aerobic conditions gives rise to considerable DDE (1).

River sediments containing DDT are reported (7) to give traces of DDT in water although these have been only 1/18,000 of the sediment levels. Absorption of DDT from water via the gills of fish has been reported (8,9) as high as 80 to 90% in the first 10 hours after application. The external mucous coating of fish was also found to concentrate DDT and aid absorption through skin. In addition to this direct absorption from water, accumulations by fish through the food chain are now well documented (10). An accumulation factor of 10,000 has been reported from water to fish, incorporating both direct absorption and food chain concentration. With this in mind, standards of 0.5 and 0.25 ppb have been suggested as maximum reasonable stream allowances (11).

In this study four areas were selected where different patterns of DDT and dieldrin use had occurred in a four-year study: 1968-71. Residues were measured in the sediments of streams and lakes and attempts were made to correlate this to accumulations in different fish species.

Study Areas

Four study areas were selected in southern Ontario. Three have soils derived from glacial deposits; the fourth is on the Canadian Shield (Precambrian).

Agricultural Area 1: This study area covered the whole of Norfolk County and parts of Elgin, Oxford, Brant, and Haldimand Counties (Fig. 1). The area is characterized by a flat topography and deep sandy loam

soils. Flue-cured tobacco was the major cash crop of the area with an annual production of 90,000 acres; this represents about 95% of the tobacco acreage in the Province. About 80% of the DDT and 8 to 9% of the aldrin-dieldrin used in Ontario have been applied to this area (Table 1). It is served by many creeks and tributaries to Long Point Bay, Lake Erie.

Agricultural—Recreational Area 2: The area consists of an agricultural marsh with deep muck soils that drain into an adjacent lake. The area is located north of the city of Toronto and supplies that city with fresh vegetables. The marsh drains by way of the Holland-Schomberg River and tributaries to Lake Simcoe where the shoreline is developed for recreation and two sizable urban centers. In the past, DDT and aldrin-dieldrin were used intensively for vegetable production. The actual quantities of each were quite small (Table 1) but the rate of application per acre was high. DDT was used around the shoreline of Lake Simcoe to control biting flies.

Mixed Agricultural—Recreational Area 3: This is a relatively large area, situated on the north shore of Lake Ontario, devoted to low-intensity mixed agricultural—recreational pursuits with some urban development. Belleville is the largest city in the area. The topography is undulating and the soils vary from shallow to deep and sandy to clay loam. Agriculture is confined mainly to Prince Edward County and the lakeshore of Northumberland and Hastings. Much of the hinterland and lakeshore is recreational. Several rivers serve the area, including the Trent, Moira, Napanee, and Salmon. They drain into the Bay of Quinte before entering Lake Ontario. About 3 to 4% of the DDT used in the Province is used in this area for fruit and some vegetable production. Aldrin-dieldrin use was confined largely to potato and vegetable production. At least one factory on the river system used dieldrin; the purpose was to impregnate carpets.

Recreational Area 4: The Muskoka lakes and rivers are popular recreational areas. This Precambrian area has little or no true soil and consists of lakes and rock outcrops with mixed deciduous and coniferous forest growing on shallow soils and detritus trapped between rocks. The whole system drains into the Georgian Bay and thence into Lake Huron. Use of DDT in this area was exceedingly small measured against use in the other areas, occurring mainly between the late 1950's and 1966 for the control of biting flies and for minor outbreaks of caterpillars and budworms.

Sediments were collected and fish were caught in gill nets from each of these four areas; samples were analyzed for DDT, its metabolites, dieldrin, and other organochlorine compounds.

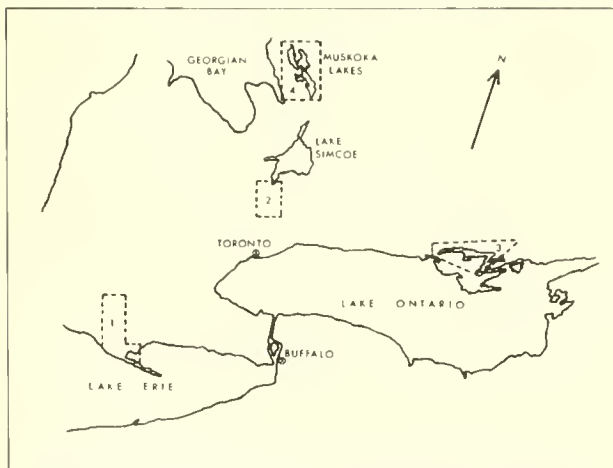


FIGURE 1.—Areas sampled for organochlorine residues in Southern Ontario, 1968-71.

TABLE 1.—Quantities of DDT and aldrin/dieldrin used in four study areas, 1968-70

INSECTICIDE	YEAR	AGRICULTURAL AREA 1 TOTAL LB, %	AGRICULTURAL— RECREATIONAL AREA 2 TOTAL LB, %	AGRICULTURAL— RECREATIONAL AREA 3 TOTAL LB, %	RECREATIONAL ¹ AREA 4 TOTAL LB	TOTAL USE IN PROVINCE, LB
DDT ²	1968	246,000 (83)	14,100 (4.8)	13,000 (4.4)	None	296,300
	1969	365,000 (79)	14,400 (3.1)	13,800 (3.0)	None	463,100
	1970	154,500 (86)	5,330 (3.0)	5,100 (2.8)	None	179,500
Aldrin—Dieldrin ³	1968	263,000 (8.1)	8,400	3,850	30	3,263,000
	1969	2,500 (9.3)	500	980	None	26,900
	1970	None	None	None	None	None

¹ Last recorded use in 1966.² Based on analysis of 5 commercial formulations, content was 75.5% *p,p'*-DDT, 18.0% *o,p'*-DDT, 6.1% *p,p'*-TDE, and 0.4% DDE.³ Over 95% of the aldrin—dieldrin used as aldrin.

Analytical Procedures

Sediments were air dried, ground, and stored until analyzed. Mineral and organic sediments were analyzed differently. Mineral sediments (25 g) were brought to within 50% of field water storage capacity and left for 24 hours. Acetone and hexane in the ratio 1:1 (250 ml) were added to the sediment and the mixture was shaken for two hours. An aliquot (100 ml) was filtered off, water was added, and the organochlorine insecticides were partitioned into hexane. Organic sediments (25 g) were blended with acetonitrile and water (2:1) for 5 minutes and an aliquot (10 g) was filtered off. The filtrate was partitioned into hexane.

Fish were held in frozen storage at -20°C . A representative sample (10 g) of the eviscerated portion was mixed thoroughly with granular anhydrous sodium sulfate (100 g) and standard Ottawa sand (25 g) by grinding in a mortar and pestle until all tissue was finely subdivided and homogeneous. This mixture was transferred to a Soxhlet extraction apparatus and subjected to exhaustive extraction with hexane for 7 hours.

Extracts from sediments and fish tissues were evaporated to dryness by rotary vacuum at 45°C . In the case of fish the percentage of fat or oil was determined gravimetrically.

A one-step Florisil column cleanup method (12) was used for the isolation of organochlorine insecticides. Florisil (60-100 mesh) activated commercially at 650°C was reheated at 135°C for a minimum of 24 hours. After cooling, the adsorbent was partly deactivated by the addition of water at the rate of 5 ml/100 g and allowed to equilibrate.

Up to 1 g of fat from fish extracts and 10 g of sediment extract were mixed thoroughly with 25 g of conditioned Florisil. This was placed on top of a second 25-g portion of prewashed adsorbent in pyrex columns (25 mm by 300 mm) fitted with reservoirs (350 ml). The entire column was eluted with 300 ml of the 1:4 dichloromethane:hexane (v/v) solvent mixture at a percolation rate of approximately 5 ml per min. Eluates were concentrated just to dryness with rotary vacuum evaporation at 45°C , the residue was redissolved in 5 ml hexane

and used for subsequent gas chromatographic analysis. All solvents employed were reagent grade chemicals which had been redistilled from glass.

Varian Aerograph Models 204 and 1200 gas chromatographs, equipped with 250 millicurie tritium electron capture detectors, were used for qualitative and quantitative assays. Operating parameters were as follows:

Column: 5 ft. by $\frac{1}{8}$ in. Pyrex packed with 4% SE-30 + 6% QF-1 on Chromosorb W, preconditioned 72 hr. at 225°C (13).
 Temperatures: column, 175°C ; detector, 200°C ; injection block, 225°C .
 Carrier gas: N_2 at 40 ml/min.

Injection volumes of 5 μl were used for both sample solutions and comparison standards.

Qualitative residue confirmation was accomplished mainly with thin-layer chromatography (TLC) using silica gel. Plates were developed with 1% chloroform in *n*-heptane, and visualized with alkaline AgNO_3 spray as the chromogenic agent. Alternatively, *p,p'*-DDT and *p,p'*-TDE were confirmed by treatment with 5% methanolic KOH (14) to produce the corresponding dehydrochlorinated derivatives and reidentification by gas chromatography. Dieldrin levels in general were too low for confirmation by either TLC or chemical conversion. Partial confirmation of dieldrin was achieved by fractionating the analysis solution on the Mills column (15), thus isolating dieldrin in the second eluate fraction.

Percent recoveries of pesticides from fish tissue were checked periodically by fortification directly into the oil obtained as the result of the hexane extraction. Averaged recoveries were: *p,p'*-DDE, 97.8%; *o,p'*-DDT, 91.2%; *p,p'*-TDE, 94.6%; *p,p'*-DDT, 89.6%; dieldrin, 89.3%. The data presented in this report do not include corrections for percent recoveries. Polychlorinated biphenyls were isolated and measured separately in 1970 and 1971 and will be reported in a second paper. Separation was attempted in 1969 but difficulties were experienced. No separation was made in 1968. The number of samples collected in 1968 were few. Later analyses showed that from those study areas included in this paper PCB's represented no more than 5% of the gas-liquid chromatography responses to DDT and TDE and no more than 1% of the DDE.

Results

SEDIMENTS

A total of 314 sediments were analyzed from lakes and rivers in the four study areas. Residues of DDT, its metabolites, and dieldrin were measured in both the collective and drainage sections of each water system. Three of the study areas were situated on deep alluvial soils (areas 1, 2, and 3) and one was located on the rocky Canadian Shield (area 4). Waterways in the alluvial soils had sediment residues slightly below those on the shield (Tables 2 A,B,C,D). The range in mean Σ DDT for the total systems varied from 56 to 96 ppb in the three alluvial soil areas and 157 ppb on the shield. In the rivers of areas 1, 2, and 3, the range in mean Σ DDT was from 70 to 101 ppb, or about twice the residue found in the receiving bays or lakes where residues ranged from a mean Σ DDT of 26 to 56 ppb. In area 4, the collecting lake system had a mean residue of 170 ppb Σ DDT; the draining river averaged 110 ppb.

Area 1: The tobacco belt is situated on deep alluvial soil and its waterways vary from very sluggish (Dedrich Creek) to quite rapid. The mean Σ DDT in the river and creek sediments varied from 19 to 146 ppb and also varied greatly on a single waterway (Table 2A). Likewise, the mean dieldrin levels varied widely from non-detectable in several bodies of water to 2 ppb in Nanticoke Creek.

The highest sediment residues of DDT were in Big Creek (125 ppb) and Dedrich Creek (146 ppb), two watercourses that were either sluggish or associated with lands intensively planted in tobacco (Table 1). Statistically, these residues are significantly higher than those in the other rivers and creeks of this system. Residues of Σ DDT in the bay (29 ppb) were considerably lower than in the river system (95 ppb), suggesting dilution and/or degradation.

In most sediments *o,p'*-DDT as well as *p,p'*-DDT were found. In the formulation of DDT used in the area the ratio of these two ingredients was 4:1. In the creek sediments *p,p'*-DDT was 5 to 8 times the residue of *o,p'*-DDT; in the bay this had increased to 25 times.

In the creek sediments, the ratios of DDE:TDE:DDT, including *o,p'* and *p,p'*-DDT, were equal; in Long Point Bay, Lake Erie, however, the ratio was 2:8:1. The increase in DDT in the bay sediment would indicate considerable degradation in the bay. There was little difference in the composition of DDE, TDE, and DDT in six creeks and rivers in the system (Table 2A).

Area 2: Water courses in this mixed agricultural—recreational area were situated on a deep alluvial soil and were associated with a considerable area of muck soil. Most tributaries were sluggish and hence sediment texture and pesticide residues varied markedly from location to location. Sandy gravel sediments had a

mean Σ DDT residue of 33 ppb and a mean dieldrin residue of 2.1 (Table 2B). Increases in residue levels appeared to be related to increased colloidal content. Organic silt had a mean Σ DDT of 170 ppb and a dieldrin residue of 3.2 ppb. The mean Σ DDT of the whole river system was 101 ppb Σ DDT and 7.8 ppb dieldrin.

In the lake sediments, which were largely sandy gravel, the mean Σ DDT was 56 ppb; no dieldrin could be detected. Movement of these two pesticides into Lake Simcoe appeared minimal as indicated by the absence of dieldrin.

Dieldrin, which was used largely for protection of vegetable crops on the marsh, appeared as residues in sediment in adjacent streams. The concentrations rose to a maximum of 137 ppb in localized sections of the river but was absent in seven Lake Simcoe sediments. DDT was used both for vegetable protection on the marsh and for controlling biting flies around the lake. Residues of DDT in the lake, therefore, were not indicative of downstream movement. When sandy gravel sediments from the rivers serving the marsh were compared with sediments of similar texture from the lake, residues of DDT and its metabolites were slightly higher in the lake sediments than in the river sediments (33 to 56 ppb). Since no clay silt or organic debris samples were collected from the lake, these could not be compared.

Some of the sediments from the river contained *o,p'*-DDT with the *p,p'*-DDT; however, lake sediments contained only *p,p'*-DDT. Although DDT was still being used in the marsh areas throughout the period of the study, its use around the lake was discontinued in 1966.

In the total river system the ratios of DDE:TDE:DDT, including *o,p'* and *p,p'*-DDT, were approximately 1:3:1, and in the lake, 1:3:5 (Table 2B). If sediments from the river are compared with those of similar texture from the lake, then ratios agree more closely. These ratios would indicate slower degradation in the sandy gravels than in the silts and organics, and suggest that lake residues were the result of local use rather than downstream movement. The most rapid degradation to TDE appeared to occur on the clay silt where ratios were 2:7:1.

Area 3: Rivers were located on shallow to deep alluvial soils and all drained into the Bay of Quinte in Lake Ontario. River sediments were quite sandy but contained considerable organic content. Mean Σ DDT residues in river sediments varied from 13 to 133 ppb with an average of 70 ppb (Table 2C). Each river showed considerable variation in residue from location to location. Mean dieldrin residues varied from 0.1 to 1.5 ppb and was 0.8 ppb for the total river system. The highest residues of DDT were found in sediment from the Trent River (133 ppb), a river associated with the

TABLE 2A.—Residues of organochlorine insecticides in sediments from rivers and creeks in tobacco belt and Long Point Bay Lake Erie (Agricultural Area 1)

LOCATION		NUMBER OF SAMPLES ANALYZED	CONTENT IN DRIED SEDIMENT, PPB ¹					DIELDRIN
			<i>p,p'</i> -DDE	<i>p,p'</i> -TDE	<i>o,p'</i> -DDT	<i>p,p'</i> -DDT	TOTAL DDT	
1. River System								
Big Creek & Tributaries	—mean	79	46.5	40.1	7.4	31.1	125.1	0.7
	—max		560.0	300.0	2.95	1205.0	2360.0	140.0
	—min		ND	ND	ND	ND	ND	ND
Dedrich Creek	—mean	19	30.5	57.4	8.9	48.9	145.7	0.9
	—max		60.0	110.0	25.0	101.0	296.0	5.0
	—min		18.0	40.0	6.0	21.0	85.0	ND
Big & Little Otter Creeks	—mean	19	13.4	9.1	1.3	16.4	40.2	1.6
	—max		28.0	14.0	4.0	27.0	72.0	9.0
	—min		1.0	0.4	0.8	4.2	6.4	0.4
Nanticoke Creek	—mean	19	17.8	22.3	3.5	14.6	58.2	2.0
	—max		55.0	134.0	3.1	25.9	218.0	12.0
	—min		5.0	9.0	0.8	3.2	18.0	ND
Forestville Creek	—mean	3	6.7	6.7	ND	13.3	26.7	ND
	—max		20.0	20.0	ND	40.0	80.0	ND
	—min		ND	ND	ND	ND	ND	ND
Grand River	—mean	19	5.1	4.2	1.1	8.8	19.2	0.9
	—max		8.0	2.0	14.0	39.0	63.0	6.0
	—min		0.4	0.5	ND	0.3	1.2	0.4
Total River System ²	—mean	158	31.4	31.4	5.5	26.5	94.8	1.0
2. Bay—Lake Erie								
Long Point Bay ³	—mean	21	5.2	20.9	0.1	2.5	28.7	0.6
	—max		12.0	165.0	3.0	8.0	188.0	12.0
	—min		ND	ND	ND	ND	ND	ND
Total River—Bay System	—mean	179	28.3	30.1	4.8	23.7	86.9	1.0

¹ ND: not detected.

² ratio of DDE:TDE:DDT in river system was 1:1:1.

³ ratio of DDE:TDE:DDT in Bay was 2:8:1.

TABLE 2B.—Residues of organochlorine insecticides in sediment from Holland Schomberg River—Lake Simcoe System (Agricultural—Recreational Area 2)

LOCATION AND TYPE OF SEDIMENT		NUMBER OF SAMPLES ANALYZED	CONTENT IN DRIED SEDIMENT, PPB ¹					DIELDRIN
			<i>p,p'</i> -DDE	<i>p,p'</i> -TDE	<i>o,p'</i> -DDT	<i>p,p'</i> -DDT	TOTAL DDT	
1. Holland Schomberg River ²								
Sand—Gravel	—mean	11	5.8	12.5	2.2	12.5	33.0	2.1
	—max		19.0	42.0	17.0	63.0	141.0	12.0
	—min		2.0	1.0	ND	ND	3.0	ND
Clay—Silt	—mean	17	22.3	68.1	3.8	14.7	108.9	13.9
	—max		118.0	450.0	15.0	56.0	639.0	137.0
	—min		Trace	4.0	ND	ND	4.0	ND
Organic—Silt	—mean	9	33.3	119.4	ND	16.9	169.6	3.2
	—max		219.0	846.0	ND	104.0	1169.0	11.0
	—min		2.0	2.0	ND	5.0	9.0	ND
Total River System	—mean	37	20.1	63.8	2.4	14.6	100.9	7.8
2. Lake Simcoe ³								
Sand—Gravel	—mean	7	5.7	16.1	ND	34.3	56.1	ND
	—max		25.0	70.0	Trace	173.0	268.0	ND
	—min		ND	ND	ND	ND	ND	ND
Total River—Lake System	—mean	44	17.8	56.2	2.0	17.8	93.8	6.6

¹ ND: not detected.

² ratio of DDE:TDE:DDT in river was 1:3:1.

³ ratio of DDE:TDE:DDT in lake was 1:3:5.

greatest intensity of agricultural, industrial, and recreational pursuits. The Bay of Quinte contained sediments with a Σ DDT residue of 26 ppb, only one third those of the river system (70 ppb), but dieldrin residues (0.5 to 0.8 ppb) were almost as high as those of the river system.

Residues of TDE predominated in sediments of all rivers of this system, suggesting that breakdown occurred under anaerobic conditions. Residues in the Bay of Quinte were similar to those in sediments from all rivers: the metabolites DDE plus TDE to DDT had a

ratio of 10:1 or greater (Table 2C). Measurable amounts of *o,p'*-DDT were found only in the Trent River, and were virtually absent from the river—bay system.

Area 4: Lakes Muskoka, Rosseau, and Joseph are contiguous bodies of water that are highly developed for recreation and are located on the Canadian Shield. Residues of DDT in the lake sediments varied from site to site; some were very low (3 ppb) and others were very high (1080 ppb). The mean Σ DDT residues for the three lakes, Muskoka, Rosseau, and Joseph, were 25, 250, 219, and 105 ppb, respectively (Table 2D). In Lake

TABLE 2C.—Residues of organochlorine insecticides in sediments from Bay of Quinte, Lake Ontario, and rivers flowing into bay (Agricultural—Recreational Area 3)

LOCATION	TYPE OF SEDIMENT	NUMBER OF SAMPLES ANALYZED	CONTENT IN DRIED SEDIMENT, PPB ¹					
			<i>p,p'</i> -DDE	<i>p,p'</i> -TDE	<i>o,p'</i> - & <i>p,p'</i> -DDT ²	TOTAL DDT	DIELDRIN	
1. River System ³								
Moira River	Organo-sand	—mean	6	13.7	24.5	3.5	41.7	1.0
		—max		9.0	123.0	ND	132.0	6.0
		—min		Trace	Trace	Trace	Trace	ND
Napanee River	Sandy-gravel	—mean	7	3.1	8.4	1.1	12.6	0.1
		—max		11.0	27.0	ND	38.0	1.0
		—min		Trace	Trace	Trace	Trace	Trace
Salmon River	Organo-sand	—mean	6	13.0	12.8	5.2	31.0	1.5
		—max		36.0	36.0	9.0	81.0	7.0
		—min		2.0	1.0	3.0	6.0	Trace
Trent River and Tributaries	Organo-sand	—mean	13	20.5	95.2	16.8	132.5	0.8
		—max		497.0	1160.0	109.0	1766.0	6.0
		—min		ND	ND	ND	ND	ND
Total River System		—mean	32	14.0	47.5	8.7	70.2	0.8
2. Bay—Lake Ontario ⁴								
Bay of Quinte	Organo-silt	—mean	15	10.3	13.3	2.1	25.7	0.5
		—max		40.0	44.0	ND	84.0	2.0
		—min		2.0	1.0	2.0	5.0	ND
Total River—Bay System		—mean	47	12.8	36.6	6.6	56.0	0.7

¹ ND: not detected.

² *o,p'*-DDT was present only in sediment from Trent River where *o,p'*-DDT was 1.5 and *p,p'*-DDT 15.3 ppm.

³ DDE:TDE:DDT ratio in river system was 2:6:1.

⁴ DDE:TDE:DDT ratio in bay was 5:6:1.

TABLE 2D.—Residues of organochlorine insecticides in sediment from Muskoka Lake System (Recreational Area 4)

LOCATION	TYPE OF SAMPLE	NUMBER OF SAMPLES ANALYZED	CONTENT IN DRIED SEDIMENT, PPB ¹					
			p,p'-DDE	p,p'-TDE	p,p'-DDT ²	TOTAL DDT	DIELDRIN	
1. Lake System ³								
L. Muskoka	Fine & coarse sand	—mean —max —min	10	8 15 2	12 56 1	5 44 Trace	25 110 3	Trace Trace ND
	Clay silt	—mean —max —min	19	108 1080 2	114 1430 1	28 370 Trace	250 2880 3	Trace Trace ND
L. Rosseau	Clay & sand silt	—mean —max —min	5	91 190 10	112 140 30	16 27 2	219 357 42	ND ND ND
L. Joseph	Clay & sand silt	—mean —max —min	5	35 130 13	64 230 ND	5 25 ND	105 385 13	ND ND ND
Total Lake System		—mean	39	71	81	18	170	
2. River Outlet ⁴								
Moon R.	Clay & sand silt	—mean —max —min	3	87 150 Trace	20 30 ND	3 5 ND	110 185 Trace	ND ND ND
N. Muskoka R.	Coarse sand	—mean —max —min	2	ND ND 68	ND ND 73	ND ND 16	ND ND 157	ND ND ND
Total Lake and River System			44					

¹ ND: not detected.

² *o,p'*-DDT not detected or in trace amounts.

³ ratio of DDE:TDE:DDT in lake system 8:9:2.

⁴ ratio of DDE:TDE:DDT in river outlet 27:7:1.

Muskoka there was a distinct difference in residue between sand and silt sediments. In 10 sandy sediments there was a mean residue of 25 ppb ΣDDT as opposed to 250 ppm for 19 silt sediments. In a traverse from shallow to deep water in the southern part of Lake Muskoka there were only slight differences in residues of sediments sampled at three depths. Sediments in the top inch contained 80 to 91 ppb ΣDDT; in the second inch the range was 13 to 36 ppb, and in the 2-4-inch depth it was 3 to 13 ppb. Residues were higher in the shallow water and declined only slightly in moderate and deep water.

The North Muskoka River which runs into the Muskoka Lakes had a coarse sand sediment in which DDT could not be detected. On the other hand, the Moon River which drains the three Muskoka Lakes into Georgian Bay, Lake Huron, had clay or silt sediments that contained a mean residue of 110 ppb ΣDDT. This river is also developed for recreation and, like the lakes, is surrounded by land that had been treated with DDT up to 1966. Since the amount of sediment moving down this river—lake system is considered very small, the largest quantity of DDT moving out of the area would be expected to be soluble. Movement of sediments later-

ally from shallow to deep water would be predicted to occur, but this would not remove residues from the system.

The ratio of DDE:TDE:DDT in the whole system indicated very low levels of the parent compound but higher residues of DDE and DDD. In the lakes this ratio was 8:9:2; in the outlet river the metabolite DDE increased to a ratio of 29:7:1 (Table 2D). Both ratios indicated considerably more breakdown of parent DDT to its metabolites than in any other system. Dieldrin and *o,p'*-DDT were virtually absent from the sediments of this system.

FISH SPECIES

A total of 1,926 sample analyses were conducted on 2,540 fish of 38 different species. In the four study areas: one agricultural, two agricultural—recreational, one recreational, there were multiple species of fish caught: 22, 20, 26, and 15, respectively. These species varied from low-fat benthic invertebrate and plankton feeders (less than 4%) to high-fat piscivores (over 7%). In each area certain species were caught predominantly in the lakes; other species were caught predominantly in rivers and streams. The following eight species were confined mainly to lake environments.

1. Alewife (*Alosa pseudoharengus*)
2. Burbot (*Lota lota*)
3. Cisco (*Coregonus artedii*)
4. Coho salmon (*Oncorhynchus kisutch*)
5. American smelt (*Osmerus mordax*)
6. Freshwater drum (*Aplodinotus grunniens*)
7. Lake Trout (*Salvelinus namaycush*)
8. Lake whitefish (*Coregonus clupeaformis*)

The following six species were caught mainly in the river environment:

1. Creek chub (*Semotilus atromaculatus*)
2. Blacknose dace (*Rhinichthys atratulus*)
3. Mooneye (*Hiodon tergisus*)
4. Brook trout (*Salvelinus fontinalis*)
5. Brown trout (*Salmo trutta*)
6. Rainbow trout (*Salmo gairdneri*)

All other species were caught in both rivers and lakes of one or more of the study areas.

The analysis data for both DDT and dieldrin are presented as tissue concentrations in the edible portion, as concentration in the extractable fat, and as total body loads of the whole fish (Tables 3, 4, 5).

Body loads are calculated from eviscerated fish concentrations and total body weights and are presented primarily for intraspecies comparison of residue accumulation with increasing size of fish. They cannot be combined as gross quantities of insecticide since the viscera may have concentrations quite different from

muscle, nor should they be used for interspecies comparison because muscle weight to body weight ratios vary considerably between species. However, they can be used as a rough index of interspecies differences in pesticide accumulation which may reflect dietary preferences and habitat.

DDT—EDIBLE TISSUE CONCENTRATIONS

In only two study areas (areas 1, 3) were fish species found with mean Σ DDT levels below 0.1 ppm (Tables 3, 4). Of these, rockbass (*Ambloplites rupestris*), bluegill (*Lepomis macrochirus*), and brown bullhead (*Ictalurus nebulosus*) were common to both areas, whereas yellow perch (*Perca flavescens*), green sunfish (*Lepomis cyanellus*), and freshwater drum were present only in area 1, and pumpkinseed (*Lepomis gibbosus*) was present only in area 3. Most were of low fat content (less than 2%) and most belonged to the family Cypriniformes. Freshwater drum with a high fat content (7.3%) contained only 0.03 ppm Σ DDT and were caught in the outer bay at Long Point, Lake Erie.

More than half the species from all study areas contained residues of Σ DDT between 0.1 and 1.0 ppm. These included the majority of the low-, medium-, and high-fat plankton feeders, the low- to medium-fat bottom feeders, and the low-fat piscivores. Alewives from area 1 and blacknose dace and emerald shiners (*Notropis atherinoides*) from area 2 were present in this group, all with quite high fat content and low residues. Most of the bottom feeders were of low fat content (1-3%) and had mean residues between 0.1 and 0.3 ppm Σ DDT, although individual brown bullhead and channel catfish (*Ictalurus punctatus*) had residues up to 0.7 ppm.

Those piscivores which had residues between 0.1 and 1.0 ppm were largely low-fat species which devoured other low-fat species. These included northern pike (*Esox lucius*) and muskellunge (*Esox masquinongy*) from area 3, and burbot from all areas. These three fish species contained residues between 0.4 and 0.6 ppm Σ DDT and were from 1.0 to 2.9% fat. Walleye (*Stizostedion vitreum vitreum*) from area 3 also fell into this category; however, they averaged only 500 g and contained 1.2% fat and 0.3 ppm Σ DDT.

About 30% of the fish species had residues of Σ DDT between 1.0 and 10 ppm in the edible tissues. Included in this group were the high-fat bottom and plankton feeders and the medium-fat piscivores. In general, the benthos feeders had fat contents over 4% and contained less than 4 ppm Σ DDT. Plankton feeders with these high residues tended to have higher contents of fat and slightly lower Σ DDT residues. Alewives (area 1) and smelt in all areas fell into this group. Many of the low- to medium-fat piscivores had residues between 1.5 and 4 ppm Σ DDT. Largemouth bass (*Micropterus salmoides*), coho salmon, and brook, brown, and rainbow trout fell into this category.

TABLE 3.—Fish species caught in 1968-71 in four study areas. 1: tobacco belt and Long Point Bay; 2: Holland—Schomberg Rivers and Lake Simcoe; 3: Bay of Quinte and its river system; 4: Muskoka Lake System

SPECIES	AREA	NUMBER OF FISH ANALYZED ¹	AVERAGE WEIGHT, G	FAT, %	CONTENT IN EDIBLE TISSUE, PPM ²				
					p,p'-DDE	p,p'-TDE	p,p'-DDT	TOTAL DDT	DIELDRIN
<i>Clupeiformes</i>									
<i>Clupeidae</i>									
Alewife	1	8	102	23.0	0.25	ND	ND	0.25	ND
(<i>Alosa pseudoharengus</i>)	3	10	53	15.6	0.64	0.16	0.53	1.33	0.042
Shad, Gizzard	3	11	310	3.3	0.184	0.148	0.094	0.426	0.009
(<i>Dorosoma cepedianum</i>)									
<i>Cypriniformes</i>									
<i>Catostomidae</i>									
Redhorse, Northern	1	3	742	1.52	0.062	0.037	0.063	0.162	0.009
(<i>Moxostoma macrolepidotum</i>)	3	16	1,151	2.00	0.124	0.070	0.104	0.298	0.004
Sucker, Longnose	4	7	454	2.14	0.075	0.024	0.122	0.221	0.005
(<i>Catostomus commersoni</i>)									
Sucker, White	1	8	196	1.02	0.076	0.013	0.025	0.114	0.009
(<i>Catostomus commersoni</i>)	2	75	456	2.2	0.098	0.048	0.047	0.193	0.010
	3	55	581	1.42	0.049	0.046	0.093	0.188	0.003
	4	107	649	1.32	0.173	0.060	0.207	0.440	0.004
<i>Cyprinidae</i>									
Carp	1	2	1,519	4.9	0.140	0.080	0.030	0.250	0.023
(<i>Cyprinus carpio</i>)									
Chub, Creek	1	5	72	3.6	0.400	0.142	0.136	0.678	0.036
(<i>Semotilus atromaculatus</i>)	2	8	60	2.4	0.041	0.036	0.037	0.114	0.003
Dace, Blacknose	1	16	2.2	4.8	0.82	0.15	0.10	1.07	0.080
(<i>Rhinichthys atratulus</i>)	2	28	1.9	6.1	0.094	0.033	0.058	0.185	Trace
Shiners, Emerald	2	473 ¹	1.2	5.94	0.202	0.140	0.044	0.386	0.022
(<i>Notropis atherinoides</i>)									
Shiners, Golden	2	182 ¹	7.3	4.11	0.471	0.411	0.161	1.043	0.094
(<i>Notemigonus crysoleucas</i>)									
Shiners, Spottail	1	10	5.2	4.17	0.154	0.084	0.144	0.382	0.018
(<i>Notropis hudsonius</i>)									
<i>Gadiformes</i>									
<i>Gadidae</i>									
Burbot	2	22	1,952	1.0	0.196	0.062	0.105	0.363	0.004
(<i>Lota lota</i>)	4	9	903	1.4	0.097	0.046	0.209	0.352	0.006
<i>Osteoglossiformes</i>									
<i>Hiodontidae</i>									
Mooneye	3	6	242	3.3	0.079	0.033	0.039	0.151	0.007
(<i>Hiodon tergisus</i>)									
<i>Perciformes</i>									
<i>Centrarchidae</i>									
Bass, Largemouth	1	2	206	7.5	2.24	0.82	0.80	3.86	0.190
(<i>Micropterus salmoides</i>)	2	12	579	3.9	0.85	0.36	0.27	1.48	0.016
	3	4	380	1.0	0.060	0.023	0.041	0.124	0.001
Bass, rock	1	15	139	1.8	0.073	0.015	0.014	0.102	0.005
(<i>Ambloplites rupestris</i>)	2	21	130	2.4	0.142	0.069	0.088	0.299	0.012
	3	38	157	1.0	0.017	0.010	0.012	0.039	0.002
	4	70	175	0.8	0.096	0.034	0.121	0.251	0.005
Bass, Smallmouth	1	18	410	2.4	0.387	0.111	0.378	0.876	0.006
(<i>Micropterus dolomieu</i>)	2	36	552	6.0	0.85	0.32	0.45	1.62	0.021
	3	24	503	2.2	1.55	0.40	0.75	2.70	0.016
	4	34	266	2.2	0.28	0.15	0.64	1.07	0.024
Bluegill	1	6	200	1.5	0.12	0.010	0.011	0.033	0.002
(<i>Lepomis macrochirus</i>)	3	5	116	1.4	0.034	0.024	0.036	0.094	0.012
Crappie, Black	1	7	97	1.4	0.056	0.041	0.036	0.133	0.015
(<i>Pomoxis nigromaculatus</i>)	3	23	169	1.2	0.071	0.052	0.063	0.186	0.001
Pumpkinseed	1	12	94	3.1	0.133	0.077	0.088	0.298	0.015
(<i>Lepomis gibbosus</i>)	2	27	51	2.9	0.175	0.221	0.189	0.585	0.023
	3	47	108	1.4	0.014	0.008	0.010	0.032	0.001
	4	8	84	1.9	0.166	0.037	0.139	0.342	0.006
Sunfish, Green	1	4	199	2.3	0.050	ND	ND	0.050	ND
(<i>Lepomis cyanellus</i>)									
<i>Cottidae</i>									
Sculpin	2	22	7.5	4.2	0.022	0.044	0.040	0.106	0.004
(<i>Cottus</i> species)									
<i>Percidae</i>									
Perch, Yellow	1	14	122	1.6	0.057	0.010	0.020	0.087	0.002
(<i>Perca flavescens</i>)	2	48	171	2.4	0.279	0.097	0.119	0.495	0.015
	3	62	154	1.2	0.082	0.040	0.072	0.194	0.001
	4	36	131	0.8	0.143	0.045	0.188	0.376	0.005
Walleye	2	33	2,706	5.3	0.87	0.24	0.61	1.72	0.022
(<i>Stizostedion vitreum vitreum</i>)	3	72	500	1.4	0.109	0.53	0.108	0.270	0.003
	4	49	1,437	2.0	0.80	0.23	1.43	2.46	0.010
<i>Sciaenidae</i>									
Drum, Freshwater	1	10	239	7.3	0.033	ND	ND	0.033	ND
(<i>Aplodinotus grunniens</i>)									

TABLE 3.—Fish species caught in 1968-71 in four study areas. 1: tobacco belt and Long Point Bay; 2: Holland—Schomberg Rivers and Lake Simcoe; 3: Bay of Quinte and its river system; 4: Muskoka Lake System—Continued

SPECIES	AREA	NUMBER OF FISH ANALYZED ¹	AVERAGE WEIGHT, G	FAT, %	CONTENT IN EDIBLE TISSUE, PPM ²				
					p,p'-DDE	p,p'-TDE	p,p'-DDT	TOTAL DDT	DIELDRIN
<i>Serranidae</i>									
Bass, White	1	12	123	3.2	0.093	0.003	0.019	0.115	0.003
(<i>Roccus chrysops</i>)	3	3	322	3.6	0.68	0.49	0.81	1.99	0.002
Perch, White	3	22	194	4.0	0.254	0.127	0.151	0.532	0.022
(<i>Roccus americanus</i>)									
<i>Salmoniformes</i>									
<i>Esocidae</i>									
Pike, Northern	2	14	993	2.9	0.307	0.104	0.157	0.568	0.006
(<i>Esox lucius</i>)	3	69	1,468	1.2	0.284	0.142	0.171	0.597	0.002
Muskellunge	3	1	1,939	2.4	0.304	0.115	0.166	0.585	0.007
(<i>Esox masquinongy</i>)	4	3	2,686	2.4	0.935	0.244	1.323	2.502	0.005
<i>Osmeridae</i>									
Smelt, American	1	13	25	2.2	0.039	0.038	0.087	0.164	0.011
(<i>Osmerus mordax</i>)	2	10	48	7.1	0.62	0.27	0.12	1.12	0.027
	3	7	36	8.6	1.28	0.75	1.20	3.23	0.043
	4	147	24	5.8	0.67	0.25	0.95	1.87	0.020
<i>Salmonidae</i>									
Cisco	2	9	101	11.2	0.32	0.29	0.67	1.28	0.046
(<i>Coregonus artedii</i>)	3	5	460	8.0	0.64	0.39	0.80	1.83	0.023
	4	6	453	5.2	1.03	0.53	2.68	4.24	0.014
Salmon, Coho	1	5	1,637	9.2	1.16	0.40	0.45	2.01	0.008
(<i>Oncorhynchus kisutch</i>)	3	4	1,068	7.1	1.22	0.26	0.71	2.19	0.064
Trout, Brook	3	1	400	5.37	1.11	0.101	0.132	1.343	0.033
(<i>Salvelinus fontinalis</i>)	4	3	121	2.45	0.109	0.027	0.082	0.218	0.010
Trout, Lake	2	21	4,318	10.2	6.32	2.05	4.07	12.44	0.162
(<i>Salvelinus namaycush</i>)	3	1	286	3.59	0.632	0.505	0.182	1.319	0.026
	4	223	2,899	10.0	6.77	2.01	13.63	22.41	0.095
Trout, Brown	1	6	122	4.77	0.94	0.15	0.38	1.47	0.047
(<i>Salmo trutta</i>)	3	2	824	5.28	1.83	0.91	0.28	3.02	0.001
Trout, Rainbow	1	9	1,757	5.14	0.76	0.21	0.25	1.22	0.068
(<i>Salmo gairdneri</i>)									
Whitefish, Lake	2	21	687	4.3	0.48	0.27	0.74	1.49	0.004
(<i>Coregonus clupeaformis</i>)	3	4	1,549	15.3	0.88	0.68	0.88	2.44	0.254
	4	21	1,632	5.6	3.08	1.52	8.34	12.94	0.054
<i>Siluriformes</i>									
<i>Ictaluridae</i>									
Bullhead, Brown	1	6	169	1.6	0.026	0.015	0.013	0.054	0.002
(<i>Ictalurus nebulosus</i>)	2	5	100	2.4	0.280	0.170	0.059	0.515	0.047
	3	33	256	1.8	0.051	0.019	0.025	0.095	0.002
	4	13	280	1.7	0.296	0.092	0.187	0.575	0.005
Catfish, Channel	2	20	118	2.9	0.095	0.080	0.036	0.211	0.004
(<i>Ictalurus punctatus</i>)	3	2	2,515	12.7	0.310	0.233	0.127	0.680	0.027

¹ Analyzed in 20-sample analyses for each species.

² The overall ratios of DDE:TDE:DDT in fish were

Area 1 5:2:3

Area 2 9:5:6

Area 3 4:2:3

Area 4 6:2:9

Only two fish species contained residues over 10 ppm. These were lake trout, a piscivore caught in areas 2 and 4, and lake whitefish, a benthic invertebrate feeder caught in area 4. The lake trout contained about 10% fat and carried the highest residues of any fish: 12.4 ppm in area 2 and 22.4 ppm in area 4. Very high residues (12.9) in lake whitefish were recorded only from area 4; weight of these fish averaged 1600 g. The tissues of fish caught in the Muskoka Lakes had the highest residues of ΣDDT of all four areas; however, the average weight of fish from those lakes was greater than the average weight of fish from other waters. Data support the finding that a buildup of concentration in ΣDDT residues is related to increased fat content, which in itself reflects the feeding habits.

The ratio of DDE:TDE:DDT varied considerably between species and between locations; however, the gen-

eral averages for all fish for areas 1, 2, and 3 were very similar. These ratios were approximately 5:2:3 in area 1, 9:5:6 in area 2, and 4:2:3 in area 3. Area 4 showed considerable difference from these; it had a ratio of 6:2:9, indicating a greater percentage of the parent compound and less breakdown to the two metabolites. These differences were reflected in most fish species to varying degrees. Of all species from the four areas, brown bullhead had the least variation in residue ratios.

DIELDRIN CONCENTRATIONS IN EDIBLE TISSUE

If the tissue residues of dieldrin are grouped into those fish containing (a) up to 0.010, (b) 0.011 to 0.100, and (c) over 0.101 ppm, then species are about equally divided into the first and second categories (Tables 3, 4).

TABLE 4.—Fish species categorized 1968-71 according to level of total DDT and dieldrin in four study areas. 1: tobacco belt & Long Point Bay; 2: Holland—Schomberg Rivers & Lake Simcoe; 3: Bay of Quinte and its river system; 4: Muskoka Lake System

STUDY AREA	INSECTICIDE	RESIDUE IN EDIBLE PORTION, PPM	SPECIES	STUDY AREA	INSECTICIDE	RESIDUE IN EDIBLE PORTION, PPM	SPECIES
1	DDT, TDE, DDE	0.00-0.10	Bass R., Bluegill, Bullhead B., Perch Y., Sheepshead, and Sunfish G.	1	Dieldrin	0.000-0.010	Alewife, Bass S., Bass S.M., Bass W., Bluegill, Bullhead B., Perch Y., Redhorse N., Salmon C., Sheepshead, Sucker W., and Sunfish G.
		0.11-1.0	Alewife, Bass S.M., Bass W., Carp, Chub C., Crappie B., Pumpkinseed, Redhorse N., Shiners S., Smelts A., and Sucker W.			0.011-0.100	Carp, Chub C., Crappie B., Dace B., Pumpkinseed, Shiners S., Smelts A., Trout Brook, Trout Brown, and Trout R.
2		1.1-10	Bass L.M., Dace B., Salmon C., Trout Brown, and Trout R.			over 0.101	Bass L.M.
		0.11-1.0	Bass R., Bullhead B., Burbot, Catfish C., Chub C., Dace B., Perch Y., Pike N., Pumpkinseed, Sculpin, Shiners E., Shiners G., and Sucker W.	2		0.000-0.010	Burbot, Catfish C., Chub C., Dace B., Pike N., Sculpin, Sucker W., and Whitefish L.
		1.1-10	Bass L.M., Bass S.M., Cisco, Smelts A., Walleye, and Whitefish L.			0.011-0.100	Bass L.M., Bass R., Bass S.M., Bullhead B., Cisco, Perch Y., Pumpkinseed, Shiners E., Shiners G., Smelts A., and Walleye.
		over 10	Trout L.			over 0.101	Trout L.
3		0.00-0.10	Bass R., Bluegill, Bullhead B., and Pumpkinseed.	3		0.000-0.010	Bass L.M., Bass R., Bass W., Bullhead B., Crappie B., Mooneye, Muskellunge, Perch Y., Pike N., Pumpkinseed, Redhorse N., Shad G., Sucker W., Trout Brook, Trout Brown, and Walleye.
		0.11-1.0	Bass L.M., Catfish C. Crappie B., Mooneye, Muskellunge, Perch W., Perch Y., Pike N., Redhorse N., Shad G., Sucker W., and Walleye.			0.011-0.100	Alewife, Bass S.M., Bluegill, Catfish C., Cisco, Perch W., Salmon C., Smelts A., and Trout L.
		1.1-10	Alewife, Bass S.M., Bass W., Cisco, Salmon C., Smelts A., Trout Brook, Trout Brown, Trout L., and Whitefish L.			over 0.101	Whitefish L.
4		0.11-1.0	Bass R., Bullhead B., Burbot, Perch Y., Pumpkinseed, Sucker L.N., Sucker W., and Trout Brook.	4		0.000-0.010	Bass R., Bullhead B., Burbot, Muskellunge, Perch Y., Pumpkinseed, Sucker L.N., Sucker W., Trout Brook and Walleye.
	1.1-10	Bass S.M., Cisco, Muskellunge, Smelts A., and Walleye.		0.011-0.100	Bass S.M., Cisco, Smelts A., Trout L., and Whitefish L.		
	over 10	Trout L., and Whitefish L.					

TABLE 5.—Accumulations of DDT and its metabolites and dieldrin in several fish species by weight class at four study areas

SPECIES	STUDY AREA	WEIGHT CLASS, G	NUMBER OF FISH ANALYZED	AVERAGE WEIGHT, G	DDT AND METABOLITES IN			DIELDRIN IN ¹		
					TISSUE, PPM	FAT, PPM	FISH, µG	TISSUE, PPM	FAT, PPM	FISH, µG
<i>Cypriniformes</i> <i>Catostomidae</i> Sucker, White (<i>Catostomus commersoni</i>)	1	0- 300	7	124	0.12	15.1	15.0	0.007	0.89	1.60
		601- 900	1	699	0.08	2.4	55.9	ND	—	ND
	2	0- 300	29	99	0.13	5.8	12.2	0.018	0.80	0.98
		301- 600	20	480	0.16	7.1	77.3	0.004	0.17	1.45
		601- 900	20	767	0.30	16.0	223.0	0.005	0.27	3.69
		901-1200	6	1,028	0.26	11.0	268.0	0.004	0.17	4.62
	3	over 1201	1	1,239	0.35	29.4	434.0	0.005	0.42	6.20
		0- 300	8	205	0.17	22.9	32.5	0.005	0.68	0.96
		301- 600	22	449	0.12	9.6	56.0	0.002	0.16	1.10
		601- 900	16	723	0.16	8.4	108.0	0.003	0.16	2.39
	4	901-1200	7	1,033	0.38	22.5	408.0	0.001	0.06	0.81
		over 1201	2	1,337	0.66	45.9	877.0	ND	—	—
		0- 300	20	225	0.09	13.7	17.1	0.002	0.32	0.56
		301- 600	27	428	0.15	13.5	70.0	0.004	0.35	1.59
	2	601- 900	20	746	0.98	52.9	743.0	0.004	0.22	3.20
		901-1200	30	1,022	0.55	37.7	560.0	0.006	0.41	5.64
		over 1201	10	1,349	0.50	29.5	658.0	0.007	0.42	8.82
<i>Cyprinidae</i> Shiners, Golden (<i>Notemigonus crysoleucas</i>)	2	0- 10	157	3.9	1.13	27.8	2.75	0.098	2.42	0.30
		11- 20	17	12.0	0.69	13.7	8.24	0.065	1.28	0.77
		21- 40	5	28.0	0.26	5.9	7.15	0.111	2.55	3.08
		over 101	3	109.0	0.04	4.5	4.64	0.035	3.64	3.85

TABLE 5.—Accumulations of DDT and its metabolites and dieldrin in several fish species by weight class at four study areas
—Continued

SPECIES	STUDY AREA	WEIGHT CLASS, G	NUMBER OF FISH ANALYZED	AVERAGE WEIGHT, G	DDT AND METABOLITES IN			DIELDRIN IN ¹		
					TISSUE, PPM	FAT, PPM	FISH, µG	TISSUE, PPM	FAT, PPM	FISH, µG
<i>Gadiformes</i> <i>Gadidae</i> Burbot (<i>Lota lota</i>)	2	0-1000	4	713	0.46	44.0	209.0	0.011	1.06	5.4
		1001-2000	7	1,469	0.32	25.3	449.0	0.005	0.40	7.4
		2001-3000	9	2,565	0.36	47.1	454.0	0.006	0.78	14.4
		over 3001	2	3,363	0.32	59.4	1,063.0	0.005	0.94	16.8
	4	0-1000	7	769	0.34	21.2	264.0	0.005	0.31	3.6
		1001-2000	2	1,374	0.39	70.2	503.0	0.008	1.43	10.8
<i>Perciformes</i> <i>Centrarchidae</i> Bass, Largemouth (<i>Micropterus salmoides</i>)	1	0- 300	2	153	3.86	51.5	740.0	0.190	4.92	34.9
	2	0- 300	5	206	0.95	25.4	201.0	0.005	0.13	1.04
		301- 600	1	343	1.23	47.3	422.0	0.005	0.19	1.72
		601- 900	4	760	1.75	40.7	1,359.0	0.006	0.14	4.55
		over 901	2	1,264	2.36	62.9	2,603.0	0.067	1.79	103.0
	3	0- 300	2	245	0.02	1.8	4.8	0.001	0.09	0.20
		301- 600	2	515	0.23	25.5	119.0	ND	—	—
Bass, Rock (<i>Ambloplites rupestris</i>)	1	0- 100	7	79	0.18	8.3	12.7	0.016	0.73	1.02
		101- 200	8	149	0.47	17.8	62.2	0.016	0.61	1.79
	2	0- 100	6	60	0.28	9.1	16.2	0.022	0.72	1.68
		101- 200	13	145	0.30	13.6	37.3	0.008	0.36	1.11
		201- 300	2	248	0.21	13.1	49.2	0.007	0.43	1.95
	3	0- 100	6	77	0.04	2.8	2.9	0.002	0.16	0.14
		101- 200	27	160	0.03	3.3	5.5	0.003	0.29	0.41
		201- 300	5	239	0.07	10.5	16.6	0.002	0.30	0.30
	4	0- 100	13	81	0.32	23.4	26.4	0.004	0.29	0.35
		101- 200	37	135	0.22	27.2	30.4	0.004	0.49	0.54
		201- 300	11	243	0.24	57.8	56.1	0.005	1.22	1.24
		over 301	9	390	0.27	52.5	110.0	0.007	1.35	2.76
Bass, Smallmouth (<i>Micropterus dolomieu</i>)	1	151- 300	10	213	0.51	32.9	109.0	0.004	0.26	0.75
		301- 450	4	352	0.88	47.5	310.0	0.006	0.32	2.11
		451- 600	2	469	2.41	81.7	1,113.0	0.020	0.68	9.02
		over 601	2	1,449	1.20	16.0	1,776.0	0.005	0.07	7.25
	2	0- 150	12	78	1.14	38.3	97.0	0.035	1.17	0.70
		151- 300	5	221	1.21	21.9	265.0	0.027	0.49	6.02
		301- 450	8	362	1.14	16.2	414.0	0.015	0.21	4.97
		451- 600	7	533	1.69	28.9	898.0	0.012	0.21	6.34
		over 601	12	959	1.86	29.9	1,761.0	0.013	0.21	11.76
	3	0- 150	4	101	0.13	7.8	11.0	0.003	0.18	0.38
		151- 300	4	229	0.10	6.5	25.0	0.001	0.06	0.33
		301- 450	7	344	0.22	13.1	77.0	0.002	0.12	0.76
		451- 600	3	525	0.33	25.2	115.0	0.003	0.23	1.73
		over 601	6	1,164	10.6	255.0	12,205.0	0.055	1.33	76.4
	4	0- 150	16	108	0.50	18.9	59.0	0.008	0.31	0.89
		151- 300	9	192	0.76	41.6	138.0	0.005	0.27	0.83
		451- 600	6	512	0.95	54.6	462.0	0.016	0.92	8.59
		over 601	3	834	4.43	258.0	5,507.0	0.181	1.05	164.0
Crappie, Black (<i>Pomoxis nigromaculatus</i>)	1	0- 150	6	84	0.13	8.4	11.1	0.017	1.12	1.04
		151- 300	1	173	0.17	17.9	29.4	0.006	0.63	1.04
	3	0- 150	15	106	0.10	12.0	8.30	0.002	0.25	0.09
		151- 300	4	204	0.03	3.1	6.21	0.002	0.20	0.43
		301- 450	4	371	0.65	21.3	272.0	—	—	—
Pumpkinseed (<i>Lepomis gibbosus</i>)	1	0- 50	3	33	1.28	27.0	39.0	0.033	0.70	4.85
		51- 100	4	81	0.08	3.1	6.0	0.017	0.68	1.33
		101- 150	4	117	0.05	1.6	5.7	0.004	0.14	0.42
		151- 200	1	174	0.05	2.2	8.7	Trace	0.02	0.07
	2	0- 50	13	29	0.94	28.8	27.0	0.036	1.10	0.94
		51- 100	13	67	0.21	8.1	14.9	0.010	0.39	0.66
		101- 150	1	107	0.76	30.6	81.0	0.010	0.40	1.09
	3	0- 50	2	20	0.07	2.7	1.2	0.001	0.04	0.01
		51- 100	11	90	0.03	2.5	1.9	0.001	0.08	0.08
		101- 150	33	118	0.03	2.4	3.3	0.001	0.08	0.12
		151- 200	1	156	0.04	2.1	6.4	0.006	0.03	0.94
	4	0- 50	2	47	0.24	26.8	11.5	0.013	1.43	0.61
		51- 100	2	61	0.28	14.1	16.2	0.006	0.30	0.34
		101- 150	4	113	0.42	17.5	47.9	0.008	0.33	0.87

TABLE 5.—Accumulations of DDT and its metabolites and dieldrin in several fish species by weight class at four study areas
—Continued

SPECIES	STUDY AREA	WEIGHT CLASS, G	NUMBER OF FISH ANALYZED	AVERAGE WEIGHT, G	DDT AND METABOLITES IN			DIELDRIN IN ¹			
					TISSUE, PPM	FAT, PPM	FISH, µG	TISSUE, PPM	FAT, PPM	FISH, µG	
<i>Percidae</i>											
Perch, Yellow (<i>Perca flavescens</i>)	1	0- 100	2	89	0.05	3.9	4.8	0.005	0.43	0.40	
		101- 200	12	125	0.09	5.6	11.5	0.002	0.12	0.22	
	2	0- 100	11	59	0.52	21.1	22.4	0.042	1.71	1.35	
		101- 200	23	137	0.41	18.0	54.6	0.006	0.26	0.83	
		201- 300	5	251	0.42	15.4	99.7	0.008	0.30	1.91	
		over 301	9	364	0.66	23.1	239.0	0.009	0.31	3.41	
	3	0- 100	13	60	0.14	12.0	8.8	0.001	0.09	0.10	
		101- 200	36	148	0.20	16.8	30.8	0.002	0.17	0.22	
		201- 300	12	254	0.25	22.4	60.5	0.001	0.09	0.15	
		over 301	1	372	0.14	9.2	52.8	Trace	0.03	0.04	
	4	0- 100	11	84	0.21	26.6	17.2	0.003	0.38	0.21	
		101- 200	23	139	0.38	50.0	54.0	0.005	0.66	0.77	
		201- 300	1	230	0.87	74.2	200.0	0.009	0.77	2.07	
		over 301	1	360	1.69	235.0	608.0	0.017	2.36	6.12	
<i>Walleye</i> (<i>Stizostedion vitreum vitreum</i>)	2	0-1000	9	605	0.32	6.8	209.0	0.016	0.34	9.0	
1001-2000		7	1,476	0.68	11.2	994.0	0.019	0.31	28.0		
2001-3000		5	2,386	4.03	64.5	9,860.0	0.025	0.40	59.1		
3001-4000		5	3,529	2.59	51.9	8,993.0	0.025	0.50	86.0		
over 4001		7	6,280	2.04	43.7	13,290.0	0.026	0.56	154.0		
3		0-1000	65	418	0.24	18.3	104.0	0.003	0.23	1.3	
		1001-2000	6	1,206	0.20	9.7	240.0	0.004	0.19	4.8	
		2001-3000	1	2,300	2.77	112.0	6,371.0	0.001	0.04	2.3	
		0-1000	26	332	0.80	72.9	352.0	0.007	0.64	3.3	
4		1001-2000	10	1,409	2.32	104.0	3,012.0	0.014	0.63	18.5	
		2001-3000	5	2,424	3.01	124.0	5,743.0	0.018	0.74	45.3	
		3001-4000	3	3,337	3.05	98.4	10,140.0	0.026	0.83	93.6	
		over 4001	5	4,902	10.4	193.0	50,140.0	0.030	0.56	144.0	
<i>Serranidae</i>											
Perch, White (<i>Roccus americanus</i>)	3	0- 150	7	116	0.36	8.6	55.6	0.028	0.68	3.12	
		151- 300	13	204	0.66	15.3	131.0	0.024	0.56	4.40	
		301- 450	2	397	0.35	27.3	136.0	0.001	0.08	0.40	
<i>Salmoniformes</i>											
<i>Esocidae</i>											
Pike, Northern (<i>Esox lucius</i>)	2	0-1000	2	625	0.09	5.8	68.0	0.002	0.14	0.61	
		1001-2000	9	1,566	0.45	14.5	702.0	0.006	0.19	7.81	
		2001-3000	1	2,782	0.850	26.6	2,365.0	0.010	0.31	2.8	
		over 3001	2	4,887	1.43	51.1	6,932.0	0.008	0.29	38.0	
	3	0-1000	25	569	0.22	28.6	125.0	0.001	0.13	0.38	
		1001-2000	30	1,467	0.75	50.1	1,122.0	0.004	0.27	4.91	
		2001-3000	7	2,286	0.73	52.9	1,723.0	0.001	0.07	3.19	
		over 3001	7	3,869	0.86	63.5	3,910.0	0.005	0.37	21.0	
<i>Osmeridae</i>											
Smelt, American (<i>Osmerus mordax</i>)	1	0- 20	4	16	0.13	7.4	2.0	0.009	0.52	0.14	
		21- 40	9	28	0.19	8.0	5.3	0.013	0.55	0.34	
	2	21- 40	4	30	0.61	11.3	18.4	0.015	0.28	0.46	
		41- 60	2	50	1.79	19.5	92.7	0.050	0.54	2.41	
		61- 80	4	66	1.29	15.5	846.0	0.033	0.40	1.80	
	3	0- 20	1	16	2.14	23.7	33.6	0.062	0.69	0.97	
		21- 40	4	31	2.81	34.6	85.1	0.040	0.49	1.27	
		41- 60	2	54	4.62	57.3	243.0	0.041	0.51	2.26	
	4	0- 20	113	17	2.00	29.5	34.2	0.023	0.34	0.39	
		21- 40	25	25	1.69	32.6	43.8	0.025	0.48	0.64	
		41- 60	6	54	1.19	38.6	51.7	0.008	0.27	0.44	
		61- 80	3	66	1.23	37.6	82.3	0.013	0.40	0.89	
	<i>Salmonidae</i>										
	Cisco (<i>Coregonus artedii</i>)	2	0- 200	9	101	0.13	1.2	130.0	0.046	0.41	4.6
		3	201- 400	2	327	0.48	9.7	152.0	0.015	0.30	4.9
			401- 600	3	548	1.02	10.3	562.0	0.028	0.28	15.5
4		201- 400	17	314	3.43	52.9	1,074.0	0.015	0.24	4.9	
		401- 600	39	485	4.27	63.4	2,126.0	0.014	0.21	6.7	
		over 601	5	730	6.21	107.0	4,543.0	0.028	0.48	21.1	
<i>Trout, Lake & Brook</i> (<i>Salvelinus namaycush</i> & <i>Salvelinus fontinalis</i>)	2	0-2000	2	1,252	4.61	50.0	7,371.0	0.095	1.03	158.0	
2001-4000		7	3,230	11.1	112.0	35,520.0	0.147	1.48	460.0		
4001-6000		7	4,570	17.6	174.0	83,420.0	0.203	2.01	908.0		
6001-8000		4	6,562	9.72	85.0	62,700.0	0.158	1.38	1,034.0		
3		0-2000	2 ²	343	1.3	30.0	457.0	0.030	0.67	10.1	
4		0-2000	67 ³	761	15.3	282.0	9,394.0	0.034	0.63	53.0	
		2001-4000	108	2,785	24.1	211.0	67,570.0	0.109	0.79	333.0	
		4001-6000	32	4,605	29.2	228.0	139,200.0	0.108	0.84	506.0	
		6001-8000	12	6,644	40.5	413.0	272,300.0	0.109	1.11	733.0	
		over 8001	8	8,731	65.8	419.0	570,600.0	0.228	1.53	1,994.0	

TABLE 5.—Accumulations of DDT and its metabolites and dieldrin in several fish species by weight class at four study areas
—Continued

SPECIES	STUDY AREA	WEIGHT CLASS, G	NUMBER OF FISH ANALYZED	AVERAGE WEIGHT, G	DDT AND METABOLITES IN			DIELDRIN IN ¹		
					TISSUE, PPM	FAT, PPM	FISH, μ G	TISSUE, PPM	FAT, PPM	FISH, μ G
Trout, Brown & Rainbow (<i>Salmo trutta</i> & <i>Salmo gairdneri</i>)	1	0-1000	10	182	1.45	31.4	192.0	0.048	1.04	6.7
		1001-2000	2	1,415	1.08	18.1	1,518.0	0.100	1.68	137.0
		2001-3000	2	2,750	1.03	18.3	2,828.0	0.070	1.24	193.0
		over 3001	1	6,400	1.35	25.1	8,640.0	0.070	1.30	448.0
	3	0-1000	2	824	3.02	57.2	226.0	0.001	0.02	0.8
Whitefish, Lake (<i>Coregonus clupeaformis</i>)	2	0- 700	10	493	0.87	23.1	486.0	0.008	0.21	4.0
		701-1400	11	762	2.18	44.9	2,000.0	0.006	0.12	4.8
	3	701-1400	3	1,238	2.36	14.8	2,921.0	0.274	1.72	341.0
		over 2101	1	2,483	2.68	20.2	6,662.0	0.193	1.45	479.0
	4	0- 700	3	566	9.49	348.0	5,758.0	0.044	1.61	23.9
		701-1400	5	1,108	16.6	327.0	17,460.0	0.050	0.98	61.6
		1401-2100	6	1,636	16.3	260.0	18,780.0	0.069	1.10	99.3
Siluriformes Ictaluridae Bullhead, Brown (<i>Ictalurus nebulosus</i>)	1	0- 150	1	95.0	0.10	7.9	0.9	0.004	0.33	0.04
		151-300	3	167.0	0.03	2.9	5.9	0.002	0.18	0.35
		301- 450	2	360.0	0.07	2.1	23.2	Trace	0.02	0.14
	2	0- 150	3	47.0	0.37	11.7	13.9	0.059	1.86	2.27
		151- 300	2	179.0	0.71	59.4	132.0	0.030	2.50	5.46
	3	0- 150	4	114.0	0.06	2.3	6.5	0.004	0.15	0.31
		151- 300	16	216.0	0.04	2.2	8.2	0.002	0.11	0.46
		301- 450	13	349.0	0.17	10.5	64.2	0.001	0.06	0.36
	4	0- 150	2	114.0	0.48	73.7	54.6	0.007	1.08	0.80
		151- 300	7	227.0	0.34	19.1	77.4	0.004	0.22	0.90
		301- 450	2	341.0	0.85	35.7	290.0	0.008	0.34	2.90
		over 451	2	568.0	1.21	68.8	704.0	0.005	0.28	3.16
Catfish, Channel (<i>Ictalurus punctatus</i>)	2	0- 100	9	90	0.18	6.9	15.9	0.003	0.11	0.31
		101- 200	10	133	0.23	7.3	32.5	0.008	0.26	0.68
		201- 300	1	233	0.33	10.5	76.0	0.002	0.06	0.47

¹ ND: not detected.

² One brook & one lake trout.

³ Three brook & 64 lake trout.

Fish with residues below 0.010 ppm were mainly bottom feeders or plankton feeders; there were also a few low-fat piscivores such as pike, muskellunge, and walleye. In areas 3 and 4 the majority of fish species fell into this low-fat category.

The fish with tissue residues between 0.011 and 0.100 ppm dieldrin included fish with higher fat content, such as the benthic invertebrate, plankton feeders, and piscivores. The majority of the feeders had residues below 0.05; the piscivores trout and salmon were above this level. The majority of fish species caught in areas 1 and 2 had residues in this middle range.

Only three species, one in each of the first three study areas, had residues over 0.1 ppm; these ranged from 0.19 to 0.26 ppm. The three species were largemouth bass in area 1, lake trout in area 2, and lake whitefish in area 3.

DDT—WEIGHT CLASSES

When fish species were divided into weight classes, a general trend of increasing tissue concentrations and increasing body loads of Σ DDT could be correlated with the increasing body weights (Table 5). This increase was not consistent for all areas or all species, but the trend was evident. In some cases, however, increasing

body weights were accompanied by decreasing tissue concentrations and either increasing body loads, e.g., pumpkinseed, or decreasing body loads, e.g., golden shiner (*Notemigonus crysoleucas*). When each weight class was well represented, the increases in both tissue concentrations and body load were readily observed; this was very evident with the fatty piscivores. Lake trout exhibited the greatest accumulations of Σ DDT.

Among the centrarchids, pumpkinseed were placed in 50-g, rockbass in 100-g, smallmouth bass (*Micropterus dolomieu*) and black crappie (*Pomoxis nigromaculatus*) in 150-g, and largemouth bass in 300-g weight classes. In area 1 the lightest pumpkinseed had the highest body loads, tissue, and fat concentrations of Σ DDT. Subsequent growth appears to have been more rapid than the accumulations of Σ DDT; there was a noticeable decline in actual extractable fat from the smaller to the larger fish where this trend existed. In areas 2, 3, and 4 this species did exhibit a general increase in body burden of Σ DDT with increasing body weight. The heaviest class contained 81 μ g of Σ DDT per 107 g of fish from area 2.

Rock bass, smallmouth bass, and black crappie all exhibited little or no increase in tissue concentrations, slight increase in the extractable fat, and marked in-

crease in the body burden of Σ DDT. The largest rockbass (mean 390 g) contained 0.27 ppm in the tissue, 52.5 ppm in the fat, and 110 μ g in the whole fish. The highest residues in smallmouth bass were from area 3 where fish with a mean weight of 1,164 g averaged 10.6 ppm Σ DDT in the tissue, 255 ppm in the fat, and 12 mg in the body.

Largemouth bass contained increasing quantities of Σ DDT in both tissues and in the total fish body. Fish weighing 1,264 g from area 2 contained 2.4 ppm in the tissue, 64 ppm in the fat, and 2.6 mg as a body load.

Among the centrarchids, rockbass, black crappie, and pumpkinseed accumulated relatively low residues compared to the largemouth and smallmouth bass. These differences were probably related to feeding habits: the two species of bass become increasingly piscivorous and consume larger fish as they themselves increase in body weight, thus accumulating high residues.

Among the bottom feeding catostomids and ictalurids, channel catfish was placed in 100-g, brown bullhead grouped in 150-g, and white sucker (*Catostomus commersoni*) in 300-g weight classes. The general trend in all three species was for slight increases of Σ DDT in fat, tissue, and body burdens. Some inconsistencies occurred, but these were probably due to the inadequate sample size of the classes. The largest sample among these species was white sucker, and even when fish averaged 1,337 g in area 3, the body accumulation was only 0.9 mg Σ DDT, much less than in the piscivorous centrarchids. Based on two of these three species, areas 3 and 4 appeared to have the greater accumulations of Σ DDT in fish tissue.

Among the percids and serranids, yellow perch was placed in 100-g, white perch (*Roccus americanus*) in 150-g, and walleye in 1-kg weight classes. Yellow perch, which was present in all four areas, exhibited the general trend of increasing Σ DDT concentrations in tissue, fat, and fish body with increasing body weight. The largest class, caught in area 4, weighed 360 g and contained 1.69 ppm Σ DDT in the tissue, 235 ppm in fat, and 0.6 mg in the fish body. Residues in these perch indicated a greater accumulation in area 4 than in the other three areas. White perch were obtained in only one area but the same general trend was observed.

Walleye were caught in three of the four study areas and in all cases the same trend of increasing tissue and fat concentration and body load occurred. The largest class weighed 4.9 kg and contained 10.4 ppm Σ DDT in the tissue, 193 ppm in the fat, and had a body load of 50 mg per fish. The difference between perch and walleye was very obvious, based on size and Σ DDT accumulations.

Burbot, a low-fat piscivore, was placed in a 1-kg weight class. It was present in areas similar to those nurturing walleye but failed to accumulate tissue residues or body loads to the same extent. A general increase did occur with the increasing body weight, however. The low-fat northern pike showed a trend similar to walleye. Among these three piscivores accumulations may reflect the species of fish consumed, as all are voracious predators and all are relatively low fat species.

The smelt, a species related to the salmonids, was placed in a 20-g weight class. It showed a marked increase in tissue and fat concentrations and body loads as the body weight increased. Smelts averaging 66 g from area 2 accumulated up to 0.8 mg Σ DDT per fish.

Among the salmonids, the benthos feeders, cisco, and whitefish were placed in 200-g and 700-g weight classes; the predaceous trout were placed in 1-kg and 2-kg classes. In all classes a marked increase in tissue and fat concentrations and in body load of Σ DDT occurred with increasing body weight. Among the ciscoes the largest fish weighed 730 g and accumulated 4.5 mg per fish in area 4; these showed greater accumulations of DDT than did ciscoes from area 3. Among the whitefish, the largest fish (2.5 kg) were found in area 4; these contained residues of almost 32 mg Σ DDT per fish. This body burden was also greater than that for fish of similar size from area 3. Among fish of the lower weight classes, whitefish had greater accumulations than fish of similar size from area 2. Brown and rainbow trout from areas 1 and 3 weighing 6.4 kg accumulated up to 8.6 mg Σ DDT. Lake trout, a fish that attained the greatest size and contained the highest fat level, exhibited the greatest increases in tissue and fat concentrations and showed the highest body burdens. Almost 0.6 g Σ DDT was found in fish weighing 8.7 kg from area 4. These voracious predators were the peak of the food chain in areas 2 and 4. Compared to the smallest yellow perch and rockbass, they had up to 500 times as much Σ DDT in tissue and fat concentrations, and up to 1000 times as much in body burden.

TISSUE, FAT, AND BODY LOADS—DIELDRIN

Dieldrin residues were several magnitudes less than the Σ DDT, but nevertheless appeared to be present in all species. Tissue and fat concentrations were not readily correlated with body weight in most benthic invertebrate and plankton feeders but were more evident in the piscivores.

Findings generally showed a correlation between increased tissue concentration and body loads with increased body weight; this was not marked in those fish of low fat content, but became more evident in high-fat bottom and plankton feeders and low-fat piscivores, and was most evident in the high-fat piscivores.

Viewed by study area, concentrations in fish tissue were greatest in area 4, even when similar weight classes were compared. Comparing weight classes, fish species in area 4 that had markedly elevated Σ DDT residues were cisco, lake whitefish, lake trout, and walleye, with slight increases in yellow perch and white suckers. In area 1, the fish species which exhibited slightly higher residues were rockbass and smallmouth bass. In area 2, pumpkinseed and bullhead had residues that were slightly higher than in fish from other areas. In area 3, only smelts had residues that exceeded those in the other three areas. Since all weight classes are not fully represented, it is difficult to predict how weight classes would have compared at all levels.

Discussion

In spite of the fact that the volume of DDT and dieldrin used in the four study areas was quite different, residues in sediment from all four areas were similar. Characteristics of each area and placement of DDT and dieldrin probably had a marked effect on these findings. In areas 2, 3, and 4, DDT was used to control biting flies and was applied close to or over water, causing fairly direct contamination of both fish and sediments. Similar results of direct application have been observed in many bodies of water (10). In area 4 the contamination of water and sediment following use was facilitated by the absence of soil, or by the very thin cover of soil in the forested areas treated. In area 1, where DDT was used in large volume, a deep sandy loam soil likely prevented direct runoff into the aquatic environment, and transportation of DDT and dieldrin in this area by physical soil movement was probably greater than in any other area studied.

The parent material *o,p'*-DDT was detected only in sediments where DDT had been used recently: namely, the tobacco belt, the rivers of area 2, and the Trent River of area 3. On the other hand, *o,p'*-DDT was absent where DDT had not been used for several years: namely, the lake in area 2, most of area 3, and area 4.

The degradation pattern of DDT primarily to TDE and in lesser degree to DDE appeared similar in all four areas, in spite of the fact that DDT was still being used in areas 1, 2, and 3 during this study but had not been used since 1966 in area 4. These ratios were 22:78; 21:79; 12:88; and 10:90%, respectively. In areas 1 and 4 the ratio of the two metabolites DDE:TDE was 1:1, probably because sediments were more aerobic than those in areas 2 and 3 (1:3 and 1:4). This supposition is supported by studies of several authors (3, 5, 6), who demonstrated increased breakdown to DDE under aerobic conditions. In areas 1, 2, and 3 DDT was applied up to and including the years of this sampling program; yet the actual level of residues in the sediments were lower than in area 4. It could be predicted from these

data that residues will take much longer to disappear from area 4 than from areas 1, 2, and 3. This slow disappearance and/or dispersion in the cooler, pre-cambrian lake environment is in keeping with the findings of Brown and Brown (16) in a subarctic environment.

Hindin et al. (7) reported traces of DDT in water coming from sediments in the ratio of 1:18,000. In a continuing study in preparation on area 1, the authors found a ratio of 1:2,400 between water and sediment for total DDT, and 1:2,700 for dieldrin. The difference between these two sets of data could be explained by differences in actual residues in the sediment and differences in the characteristics of the body of water. Nevertheless, sediments act as reservoirs of organochlorine insecticides which are constantly available for incorporation into tissues of bottom living organisms. Although two studies (8,9) have demonstrated that fish can remove 80 to 90% of the DDT from water by gills, a third study (17) has shown that 10 times more DDT was accumulated from the food chain than directly from water during the period of observation. Sediment residues can therefore be either ingested directly by invertebrates or bottom feeders or indirectly by transfer through the water phase.

In this study the ratio of metabolites to DDT in a pooled average of all fish by area was 7:3 in areas 1, 2, and 3, and 9:11 in area 4. The first three areas were almost identical and could be closely correlated with that ratio in the sediment; however, area 4 was distinctly different both with respect to fish and sediment. These differences may be related to the limnological characteristics of this deep, oligotrophic lake system which varies markedly from the shallow, eutrophic conditions prevalent in the other areas. Ratios of the metabolites DDE and TDE in fish from the four areas were 2:1 in areas 1, 2, and 3; and 3:1 in area 4. These ratios are almost the reverse of those found in sediments.

Comparing the mean pesticide concentration by area, a multiplication factor from the lowest to the highest residue in fish ranged from 100 to 450. If weight class were taken into consideration, residue or magnification of the tissue from smallest to largest fish regardless of species varied from 130 to 530. If the body load of DDT in micrograms per fish is considered, an increase from the lowest to the highest ranged from 10^3 to 33×10^3 . Dieldrin residues in tissue by a similar comparison rose by factors of 60 to 200. When weight class was included, the total accumulation per fish increased by multiples of 3 to 5×10^3 .

The concentration of both DDT and dieldrin in sediment (dry-weight basis) was about equivalent to the residue found in the tissue of the lowest fish species. The mean residues of Σ DDT in sediment and fish with the lowest tissue concentrations for the four areas were: area

1: (agricultural): 0.07 and 0.03 ppm; areas 2 and 3 (agricultural—recreational): 0.09 and 0.04 ppm, and 0.06 and 0.02 ppm, respectively; area 4 (recreational): 0.16 and 0.22 ppm.

The relationships established in this study for Σ DDT between sediment and fish, with further data on water, provided a ratio of sediment to water of 2,400:1; the ratio of sediment to low-fat fish was about 1:1. The ratio of lowest fish to highest fish was between 10^3 and 10^4 , which gives a ratio of water to highest piscivores of $1:10^6$ or 10^7 . A similar ratio could also be applied to the accumulation of dieldrin residues.

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Relations of the Brown Pelican to Certain Environmental Pollutants¹

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ABSTRACT

Nearly all brown pelican eggs collected from 13 colonies in South Carolina, Florida, and California in 1969 and from 17 colonies in South Carolina and Florida in 1970 exhibited eggshell thinning. Of the 100 eggs analyzed for residues of pollutants, all eggs contained measurable quantities of DDE; most eggs contained measurable quantities of p,p'-DDD, p,p'-DDT, dieldrin, or PCB's (polychlorinated biphenyls). All eggs contained measurable quantities of mercury. DDE appears to have been responsible for virtually all the eggshell thinning. There is strong evidence that DDE played a major role in lowered reproductive success in South Carolina and California, and this pollutant appears to be intimately related to the population decline in South Carolina. Other pollutants, particularly dieldrin, may have had deleterious effect on reproductive success in South Carolina. Carcasses of pelicans collected by shooting in Florida and South Carolina in 1970 varied in residue load according to age and geographic location. Birds under 1 year of age contained smaller quantities of residues than did birds 1 year or older.

Introduction

The brown pelican (*Pelecanus occidentalis*) has experienced drastic population declines in much of its range in the United States. The first indication of population decline was noted in the early 1960's when the large breeding population of the eastern brown pelican (*P. o. carolinensis*) in Louisiana was extirpated (1). In South Carolina, the population declined from over 5,000 breeding pairs to approximately 1,000 pairs by the late 1960's (2, 3). In California, near total reproductive failure has occurred in the California brown pelican (*P. o. californicus*) during the past several years (4, 5). The population in Florida has remained relatively stable over the past 5 years (6, 7; also: L. J. Williams,

personal communication). The agent or agents responsible for the disappearance of the Louisiana population remain unknown, but the catastrophe in California has been attributed to the DDT group of insecticides, particularly DDE (4, 8, 9, 10).

The objectives of this study are (a) to determine the relation of certain environmental pollutants to population trends of the eastern brown pelican in Florida and South Carolina, and (b) to examine the relation of these pollutants to reproductive success in South Carolina. Additional residue data are included from the California brown pelican. Eggs and birds from which residue data were determined and herein reported were collected in 1969 and 1970.

Sampling Procedures

Brown pelican eggs were collected in 1969 from one colony in California (10 eggs), two colonies in South Carolina (49 eggs), and ten colonies in Florida (81 eggs). The eggshell measurements of the eggs collected in 1969 from the southeastern colonies were reported previously (3); and some of the residue data for the eggs from California were previously reported (11). In 1970, an additional 186 eggs were collected: 146 from 15 colonies in Florida and 40 from two colonies in South Carolina (Fig. 1). The eggs were wrapped in aluminum foil and frozen within several days of collection. Shell thickness (shell and shell membranes) was measured at three sites on the waist with a micrometer graduated in units of 0.01 mm. The thoroughly dried shells were weighed to the nearest 0.0001 g.

When feasible, one egg was taken from each of 10 nests in each Florida colony; at least 20 were collected from each South Carolina colony. Except for a few instances in 1969, eggs were collected from nests that had no young; eggs in all stages of incubation, both fresh and

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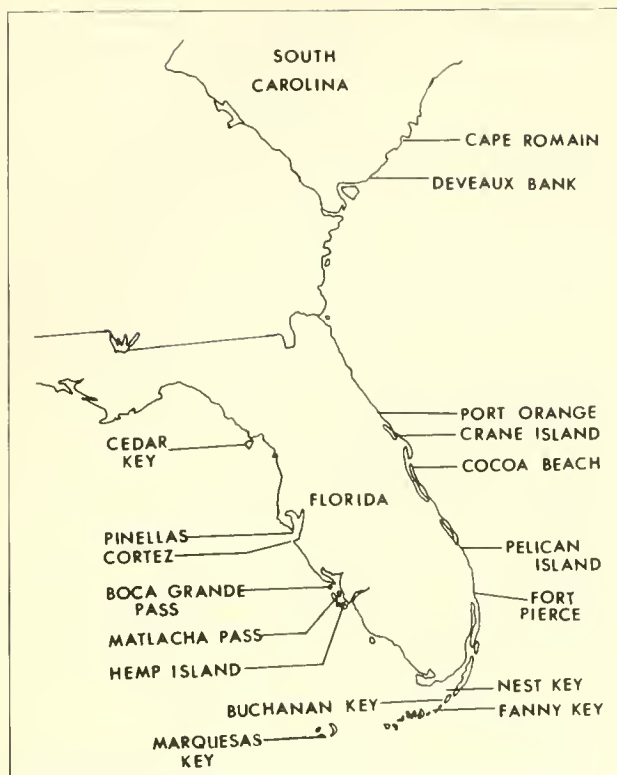


FIGURE 1.—Colony sites where brown pelican eggs were collected in 1970.

added, were collected. In 1969, the eggs were collected in late May or early June except in the Deveaux Bank colony where they were collected in late July. In 1970, eggs were collected in February in three Florida Bay colonies; other colonies were visited in late April or early May. An attempt was made to sample as wide a segment of each colony as possible. The South Carolina colonies contain ground-nesting birds with only a few nests in low shrubs; this facilitated uniform sampling of the colony. It was more difficult to obtain a uniform sample in some Florida colonies because the pelicans nest in mangroves and other trees.

Pelicans were shot in three areas in Florida and in one area in South Carolina in 1970. Birds were collected in early June in Florida Bay and from July 7 to July 23 in the other areas. Pelicans were selected to represent a varied sample of age and sex. In one instance, a sick individual was collected; all other pelicans appeared normal.

In South Carolina, reproductive success was determined by directly counting or estimating the total number of nests and the total number of young fledged from those nests. Nests were counted on Marsh Island in 1969 and 1970 and on Deveaux Bank in 1970. The number of fledged young in each year was estimated as was the count of nests on Deveaux Bank in 1969.

Chemical Analyses

Eggs were analyzed individually for residues of organochlorine pesticides and their metabolites, polychlorinated biphenyls (PCB's), and mercury. The content of each egg was homogenized in a mixer; a 20-g aliquot was removed for pesticide and PCB analysis. A 5-g aliquot was removed for mercury analysis from each egg collected from South Carolina in 1970. The 20-g aliquots were ground with anhydrous sodium sulfate and extracted for 7 hours with hexane in a Soxhlet apparatus. Extracts were cleaned up by acetonitrile partitioning, and half were saved for PCB analysis. For insecticide analysis, residues in the cleaned extract were separated and removed in four fractions from a silica gel thin-layer plate (12). Each sample fraction was analyzed by electron capture gas chromatography on columns consisting of either a 3% OV-1 on Chromosorb W H. P. on 80/100 P, a 3% OV-17 on Gas Chrom Q on 100/120, or a 3.8% UCW-98 on Chrom W H.P. 80/100. DDT and metabolites in Fractions III or IV were confirmed on a column of 3% XE-60 or 3% QF-1 on Gas Chrom Q 60/80. PCB residues were identified and measured semi-quantitatively by a method involving thin-layer chromatography (13). The average recovery of the chlorinated insecticides and their metabolites from fortified eagle tissue ranged from 75 to 112%.

The California eggs, after freeze-drying, were analyzed for lead and total mercury by PPB, Incorporated, Columbia, Missouri (now defunct). The lead analysis was done on an atomic absorption spectrometer; a tantalum boat technique was used to increase sensitivity. The 1969 eggs from Florida and South Carolina were analyzed for total mercury by PPB, Incorporated, and Gulf General Atomic, Incorporated; quantification and identification of mercury in the freeze-dried eggs were made by multichannel gamma ray spectrometry. The analysis for mercury for the Florida Bay eggs and the analysis for lead in eggs not from California were conducted by the Environmental Trace Substances Center, Columbia, Missouri.

The mercury analysis of the 10 eggs collected in South Carolina in 1970 was for total mercury by a method developed by R. S. Christensen of the WARF Institute, Inc., Madison, Wisconsin (personal communication). The procedure involves acid digestion of the tissue and extraction of the mercury from the liquid digest with dithizone (14). Mercury determination was made on the dithizone extract by flame atomic absorption spectrophotometry using a sampling boat technique (15). The average recovery from tissue samples fortified with both inorganic mercury compounds was 91%.

The organochlorine residues of the pelican carcasses (body minus skin, head, feet, and gastrointestinal tract)

collected in 1970 and the eggs collected from Florida Bay in 1970 were analyzed by a method different from the procedure used for the other 80 eggs. Use of this method permitted analysis for mirex, which was not feasible using the method described above. The usual Soxhlet extraction with hexane was made on a 10-g aliquot of pelican carcass. An aliquot containing ≤ 0.4 g lipid was cleaned up by column chromatography using the following procedure: Florisil, previously washed and recalcined at 1250°F according to the method of Hall (16), was partly deactivated with water (1-1.5%) to allow dieldrin to elute with the other pesticides and PCB's. A 19-mm-inside-diameter column containing 21 g of the treated Florisil topped with $\frac{1}{2}$ in. sodium sulfate was prewashed with 50 ml hexane; the sample was added to the column and eluted with 200 ml of 6% ethyl ether in hexane. The Florisil eluate was evaporated to 20 ml and a 5-ml aliquot was transferred to a silicic acid column.

The reagents and apparatus used for the silicic acid column procedure were those specified by Armour and Burke (17) but modifications were made in collecting the eluants. The petroleum ether eluate was collected in two separate fractions of 100 and 300 ml, followed by 200 ml of the polar eluate (1% acetonitrile, 19% hexane, and 80% methylene chloride). The three fractions were concentrated and analyzed by gas chromatography (GC).

Using this procedure, mirex was collected in the first 100 ml of petroleum ether eluate. PCB's and DDE were collected in the second fraction; a previous study (18) showed that PCB preparations (Aroclor 1248, 1254, or 1260 as reference) do not interfere in the quantitative determination of DDE unless the ratio of PCB to DDE exceeds 10:1. The third fraction contained the rest of the pesticides.

Each fraction was analyzed on a Hewlett Packard 5750 gas chromatograph using a column of 4% SE-30/6% QF-1 on Supelcoport 100/120. Pesticides were quantitated by peak height; PCB's were estimated by comparing the total GC peak heights of PCB's in samples with that of Aroclor 1254. Recoveries from eagle tissue spiked with the organochlorine pesticides and 1254 ranged from 90 to 109%. The polar eluate of some samples was zoned by thin-layer chromatography (12) and run on a 3% SP 2401 column to confirm certain pesticide residues. PCB residues in most samples were confirmed by the thin-layer method of Mulhern et al. (13).

The minimum measurable quantity was 0.05 $\mu\text{g/g}$ for most pesticides. The pelican egg residue measurements were not corrected for recovery values. Residues are expressed on a fresh wet-weight basis. We found that certain external egg measurements (length by breadth) were significantly correlated ($P < 0.01$) with the weight

of the contents of fresh eggs (1). The resulting regression equation was used to convert weight of the contents of all eggs to a fresh wet-weight basis (19). For purposes of comparison with other research, the contents of freshly laid brown pelican eggs contained an average of 84% moisture (determined by lyophilization) and 4.4% lipids. The percentage lipids remained essentially uniform until the embryo reached late development. At this stage (two-thirds or more development) the contents (adjusted to fresh wet weight) contained an average of 3.1% lipids, a significant drop ($P < 0.01$; Student's *t*-test) from that found in the fresh egg. Romanoff (20) found a decrease of one-third of the lipid content of the hen's egg from laying to hatching.

A trace value for the pesticides or their metabolites, lead, or mercury was considered to be $< 0.1 \mu\text{g/g}$, and a trace value for the PCB's was considered to be $< 1.0 \mu\text{g/g}$. In order to quantify trace values and zero values (no residue detected) for statistical tests, the mean of these values was considered to lie halfway between the level of sensitivity and zero; each of these values was assigned this mean. Statistical analyses of the egg data were performed after logarithmic transformation of the residues. Residues in the 20 eggs collected from colonies in Florida Bay were not used in any of the statistical analysis except to show the mean and median for each colony.

Results and Discussion

RESIDUES

Measurable residues of *p,p'*-DDE were found in the 100 eggs analyzed; residues of *p,p'*-DDD, *p,p'*-DDT, dieldrin, and PCB's exceeded trace values in most eggs (Table 1). Measurable quantities of mercury were found in the 80 eggs analyzed for this metal. There was a great deal of variability in the DDE residues: California eggs (1969) had a geometric mean of 71 $\mu\text{g/g}$; residues in South Carolina eggs (Cape Romain) varied from 4.45 $\mu\text{g/g}$ in 1969 to 2.83 in 1970; and residues in eggs from the Florida colonies (1969) averaged 0.69 to 2.48 $\mu\text{g/g}$ DDE. Residues of DDE in eggs collected in 1969 from four geographic areas: Florida Gulf Coast, Florida Atlantic Coast, South Carolina, and California, were significantly different ($P < 0.05$) from one another (Table 2). California eggs contained significantly more DDE and DDT than the eggs from the other areas; South Carolina eggs contained significantly more dieldrin. The highest levels of DDD and PCB's were also found in the South Carolina eggs; these residues were significantly higher than in the other areas except California. Excluding mercury, the lowest residues were found in the Florida Gulf Coast eggs. The lowest level of dieldrin was found in the California eggs, and the lowest level of mercury was found in the South Carolina eggs. The DDE:PCB ratio varied from 0.3:1 in the Marquesas Key Colony to 20:1 in the Anacapa Colony. All Anacapa eggs contained small residues

of *o,p'*-DDT and *o,p'*-DDD; it is extremely rare to find these isomers in tissues of vertebrates (11). Lead residues in the eggs were small; maximum residue was 0.17 $\mu\text{g/g}$. Most egg residues were usually positively intercorrelated ($P < 0.05$) (Table 3). The exception was mercury which was negatively correlated with most of the other residues. The negative correlations probably occur as a result of a dissimilar pattern of mercury contamination from the organochlorines. The buildup of mercury in the food chain is likely to be of a different nature and of a lower magnitude than that associated with the organochlorines because most of the major forms of mercury in the ecosystem apparently are not lipid soluble. There were some differences between States in the correlations between egg residues.

The positive relationships between DDE and the other organochlorines were usually significant. To a lesser extent, this was also true of DDD and the PCB's. In contrast, the relationship of DDT to the other organochlorine residues, although positive, was usually not significant. The exception was in the California series where there was a significant positive relationship between DDT and DDE and between DDT and DDD. In the South Carolina eggs, the negative relationships between mercury and several of the organochlorines were significant. Although the full meaning of the relationship between residues is not clear, it is known that DDT is metabolized in the hen's egg to some extent (21). Consequently, there may be a breakdown from DDT to DDD in eggs containing dead embryos since this metabolism occurs in avian tissue after death (22). Thus the relationships in Table 3 may not necessarily be representative of those found in freshly laid eggs. However, it seems certain that the biological magnification of certain organochlorines (*p,p'*-DDE, *p,p'*-DDD, dieldrin, and PCB's) resembles a group effect in the pelican egg. That is, organochlorine residues tend to increase or decrease together, although the level of change for each chemical may be of a different magnitude.

Residue data are included for only one pelican found dead in the field (Table 4). This particular bird was observed in tremors on May 8 in the yard of a house located on Plantation Key, Florida. It was found dead on May 9. The cause of death is not known because no indications of serious disease or unduly high residues of pollutants were found. A hemolytic *Staphylococcus* was isolated from the heart blood, but the significance of this isolation was not determined. The carcass contained an array of residues, but these were lower than some of the residues from carcasses of pelicans collected in 1970 (Table 5). The residues in the brain were lower than those considered to be indicative of death from dieldrin (23), or from DDT or metabolites (24, 25).

Residues in the carcasses of birds collected in 1970 were quite variable, but all carcasses contained measurable residues of DDE and all contained at least a trace of

PCB's (Table 5). The birds represented a wide range of age and both sexes; thus it would have little meaning to calculate the geometric mean or determine the median residue. The immature birds (1 to 2 years of age) and adult birds (3 years and older) always carried higher residues than did the young of the year. The age at which the brown pelican attains adult plumage is not definitely known; the age brackets for immature and adults pelicans are approximate. There was an indication that residues increased with age in young of the year, but this was not universally true.

Eggshell thicknesses of eggs from Florida Bay were near normal, and residues of DDE in these eggs averaged near 1 $\mu\text{g/g}$, which was generally lower than averages found in eggs from other colonies. Residues were much lower in adult and immature pelicans from Florida Bay than were those detected in birds from the other areas. Perhaps most of these birds spend the entire season in Florida Bay where they probably are exposed to a lower level of contamination. However, we currently have no explanation for the similar magnitude of residues in young of the year from all areas. The pelicans may winter in areas other than those in which they breed (26). This is particularly probable of pelicans breeding in South Carolina; most of the birds winter on the east coast of Florida, a few winter in South Carolina, and some winter in Cuba and other southern localities. The east coast and west coast pelicans in Florida generally remain apart from one another. There are no data for birds banded in Florida Bay. Except for birds from Florida Bay, there are no clearcut differences, by locality, in the magnitude of residues in the carcasses; this may be due partly to the small sample size. Residues of DDE and PCB's in carcasses of several of the immature and adult birds seem unduly high, but there is no current basis for associating these residues with adverse biological effects.

EGGSHELL MEASUREMENTS

To determine whether the brown pelican eggshell thickness measurements varied with time in the pre-1947 sample, the Florida thickness measurements were grouped by decade from 1890-1899 to 1930-1939. Thickness by decade ranged from 0.549 mm in the 1920-1929 sample to 0.568 mm in the 1910-1919 sample; an analysis of variance indicated no significant difference ($P > 0.05$) between decades (Table 6). Thus all the pre-1947 measurements were combined to compare with the 1969 and 1970 thickness data. There were insufficient measurements in the pre-1947 thickness measurements from South Carolina with which to compare decade differences. Anderson and Hickey (27) reported the eggshell thickness of 11 species of birds by decades up to 1939; only one species exhibited significant decade differences.

Comparisons of the 1969 mean shell thickness with the 1970 mean shell thickness of eggs from each of nine

colonies in Florida and two colonies in South Carolina showed that the yearly means were significantly different ($P < 0.05$, Student's *t*-test) in only one colony: Boca Grande Pass. The thickness of shells from this colony was 0.559 mm in 1969 compared to 0.517 in 1970.

The eggshell thicknesses of eggs collected in 1970 in Florida and South Carolina were similar to those obtained in 1969 (Tables 7 and 8). The mean eggshell thickness of the Florida eggs collected in 1970 was 0.512 mm, a decrease of 8.1% from the pre-1947 measurements. As in 1969, the thinnest eggs from the southeastern colonies were found in South Carolina; the thickness was approximately 17% less than that of shells in the pre-1947 museum collections. In each State mean eggshell thicknesses for both 1969 and 1970 were significantly less ($P < 0.05$) than the pre-1947 eggshell thickness; however, there were no significant differences between the 1969 and 1970 means (Table 8).

The 17% eggshell thinning observed in South Carolina was associated with subnormal reproductive success. We saw several instances of extremely thin eggshells in the South Carolina colonies in both years. Usually, the entire clutch exhibited the extreme thinning, and all the eggs were broken in some nests. The thinnest egg was collected from the Cape Romain colony (33.6% thinner than the pre-1947 mean); it apparently had been laid only hours before collection. Crushing of thinshelled eggs early in the incubation period provides an unknown degree of bias favoring collection of eggs with thicker eggshells.

The mean eggshell thickness of eggs from the Florida Bay colonies varied from 2 to 6% less than the pre-1947 mean. Eggshell thinning in the other two major geographic areas of Florida was somewhat greater: 5 to 8% in the Gulf Coast eggs and about 11% in the Atlantic Coast eggs. It is unknown whether reproduction is impaired when shell thinning reaches 11%.

In this study, we have used eggshell thickness or percentage of pre-1947 thickness in preference to eggshell weight or the thickness index. Each of the four measurements is significantly correlated ($P < 0.01$) with the others (Table 9). Therefore it follows that any one of the measurements may be used. An important reason for using thickness is that this is the only measurement that can be taken from crushed or hatched eggs. The reason for using the percentage of the pre-1947 thickness is that thickness measurements for different subspecies or their groups can be combined for analysis of the data; this is not possible using thickness alone due to inherent differences by geographic area in the pre-1947 measurements. It is also possible to compare the relationships between thickness and residue level in eggs of different species. Anderson and Hickey (27) found a significant correlation between eggshell weight and thickness index in each of 10 species of birds.

RELATIONSHIP BETWEEN RESIDUES AND EGGSHELL MEASUREMENTS

Most residues in brown pelican eggs have been shown to be significantly correlated with the eggshell measurements (9). Mercury was usually positively correlated with the thickness measurements, and in one instance the correlation coefficient was significant ($P < 0.05$). By use of stepwise regression, DDE was the only residue that consistently accounted for a significant amount of variability in the three eggshell measurements (9). The intimate relationship between DDE and eggshell thinning in the pelican is most impressive, especially considering that the data are taken from wild birds (Fig. 2). Of course, there are natural factors that may influence the thickness of birds' eggshells that were not considered in the analysis. The eggshell thinning potential of certain other pollutants that may occur in pelican eggs has not been thoroughly tested and analytical methods for detecting them are limited. DDE has significantly altered the shell thickness of eggs laid by certain captive birds (28, 29, 30, 31), and this compound is universally distributed in wild birds. Thus it is the most likely candidate to be responsible for most of the thinning of eggshells of wild birds. Lead, mercury, and PCB's have been suggested as the shell-thinning agents in the California pelicans; however, residues of mercury and PCB's are no higher in the California eggs than in the southeastern eggs. In all eggs, levels of lead were very low in these eggs and it seems unlikely that lead is responsible for the thinning. Connors et al. (32) compared concentrations of nine metals in pelican tissue from Florida and California; residue levels were similar in both areas except that mercury levels were higher in Florida. They considered it unlikely that eggshell thinning and reproductive failure in the brown pelican in California were induced by any of the nine metals.

The rate at which DDE thins eggshells of the brown pelican is greatest when the residues are small (8). This relationship is essentially linear on a logarithmic scale.

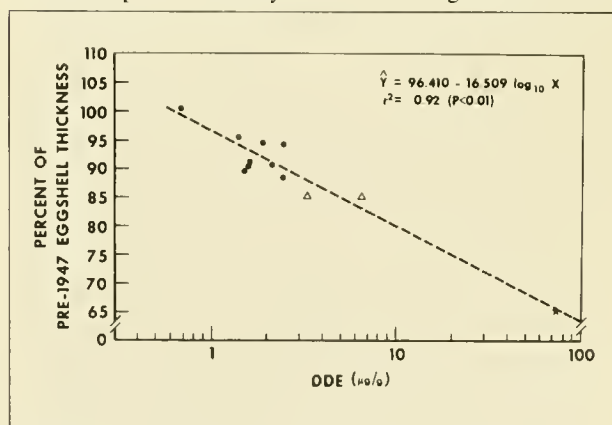


FIGURE 2.—Association of DDE residues in brown pelican eggs from nine collections in Florida [●], two colonies in South Carolina [Δ], and one colony in California [★] with percent of pre-1947 eggshell thickness.

The calculated no-effect level is 0.5 $\mu\text{g/g}$ of DDE in the egg, although this level may actually be lower than calculated. Thinning reaches 4% at 1 $\mu\text{g/g}$, 15% at 5 $\mu\text{g/g}$, 20% at 10 $\mu\text{g/g}$, and 40% at 100 $\mu\text{g/g}$. The logarithmic relationship of DDE to thinning also appeared to be present in the double-crested cormorant (*Phalacrocorax auritus*) (33) and the prairie falcon (*Falco mexicanus*) (34). Further research is required to define the mathematical relationships of residues to other biological effects.

EFFECTS OF RESIDUES ON REPRODUCTIVE SUCCESS

Data are available from experimental studies that indicate the relationship between eggshell thinning and reproductive success. In experimental studies with DDE in mallards (*Anas platyrhynchos*) and black ducks (*Anas rubripes*) where thinning was less than 18%, increased embryonic mortality, and increased egg breakage were found; both factors resulted in decreased reproductive success (28, 29). Other factors associated with eggshell thinning that are found in wild birds are increased egg eating by the parents (35), decreased clutch size, and increased embryonic mortality (36). It seems likely that DDE is one of the principal agents responsible for the decreased productivity of the pelican in South Carolina (Table 10). However, there are three other pollutants that may have adversely influenced reproductive success. The major suspect is dieldrin. Experimental studies have demonstrated that, even at high residue levels in the egg, dieldrin has little or no effect on eggshell thickness in the mallard (37) or ring-necked pheasant (*Phasianus colchicus*) (38). However, dieldrin has affected reproductive success in experimental ring-necked pheasants (39).

The mean dieldrin level in both South Carolina pelican colonies was near 1 $\mu\text{g/g}$ in 1969. The dieldrin levels in the Cape Romain colony dropped to 0.62 $\mu\text{g/g}$ in 1970. Most of the dieldrin levels in eggs were near the levels thought to cause a lowered reproductive success in the Scottish golden eagle (*Aquila chrysaetos*) (40, 41) and in bald eagles (*Haliaeetus leucocephalus*) in Maine (42). Species are not equally sensitive to dieldrin; reproductive success in the gallinules (*Porphyrio martinica* and *Gallinula chloropus*) seemed to be normal even though dieldrin levels in eggs averaged near 4 $\mu\text{g/g}$ (43, 44). Potts (10) found egg breakage and some evidence of lowered reproductive success in the shag (*Phalacrocorax aristotelis*); dieldrin represented the only chlorinated hydrocarbon pesticide present in appreciable quantities. Potts concluded that much of the egg breakage was caused by polygyny. Apparently, adverse reproductive effects in the shag did not appear until dieldrin residues in the egg exceeded 2 $\mu\text{g/g}$ of dieldrin. These studies demonstrate that different species may respond quite differently to the same toxicant. Judging from the extreme sensitivity of the brown pelican to DDE-induced eggshell thinning, one would

suspect that the pelican is also extremely sensitive to dieldrin. However, further study is needed to define this relationship.

PCB's constitute another group of pollutants that may be adversely affecting reproductive success, but this has not been demonstrated in wild birds. The experimental evidence regarding the ability of PCB's to thin eggshells and adversely affect reproductive success is conflicting. In one study with chickens, significant adverse effects on productivity occurred when the chickens were fed 10 and 100 $\mu\text{g/g}$ of Aroclor 1242 or 100 $\mu\text{g/g}$ of Aroclor 1254. The effects were reduced egg production and hatchability and thinning of eggshells (45). In a study with mallards, no eggshell thinning or other adverse reproductive effects were observed when Aroclor 1254 was included in the diet at 25 $\mu\text{g/g}$; bobwhite quail showed no shell thinning or other adverse reproductive effects when fed 50 $\mu\text{g/g}$ of Aroclor 1254 (46). Pheasants given a 50-mg capsule dose of Aroclor 1254 weekly for 17 weeks produced fewer eggs than did controls, and significantly more young died while pipping the shell. Chick survival was also adversely affected by Aroclor 1254, but neither eggshell thickness nor fertility was affected (47). In ring doves (*Streptopelia risoria*) fed 10 $\mu\text{g/g}$ of Aroclor 1254 for two years, embryonic mortality and chromosomal alterations were found only in the second year (48). Although not all the PCB preparations have been tested to determine whether they will thin eggshells, it appears unlikely that they are contributing significantly to thinning in the pelicans (9). However, the possibility still exists that the PCB's may also be involved in the lowered reproductive success.

Mercury also may be adversely affecting reproduction in the pelican. In a study with ring-necked pheasants fed 10 $\mu\text{g/g}$ of ethyl mercury p-toluene sulfanilide, egg production was decreased by 50-80% and embryonic mortality was increased in the few eggs that were laid. Eggshell thickness was not significantly reduced in the birds treated (49). In pheasants fed grain treated with methylmercury dicyandiamide, reduced egg production and reduced hatchability occurred. A large number of eggs without shells were found in some groups, but eggshell thickness was not measured (50). In another study in which pheasants were fed grain treated with methylmercury dicyandiamide, reduced hatchability was noted after nine days on dosage; eggshell thickness was not measured (51). In these studies with pheasants, the mercury level in the eggs ranged from 1.3 to 2.0 $\mu\text{g/g}$ (51), from 0.9 to 3.1 (48), and from 0.5 to 1.5 $\mu\text{g/g}$ (50). In a study with Coturnix quail fed 1 $\mu\text{g/g}$ of mercuric chloride, the eggshell thickness was significantly lower than in controls, yet the eggs contained only 0.06 $\mu\text{g/g}$ of mercury. At the same time, egg production, hatchability, and fertility were unaffected even at a dietary level as high as 8 $\mu\text{g/g}$ (52).

In wild birds, Vermeer (53) found mercury levels of 0.5 to 2.0 $\mu\text{g/g}$ in eggs collected from successful herring gull clutches; an egg from each of two clutches that produced no young contained 1.3 and 2.4 $\mu\text{g/g}$. Six of the 21 pelican eggs from South Carolina contained 0.5 $\mu\text{g/g}$ or more of mercury, a level associated with adverse effects on reproductive success in one of the experimental studies with pheasants. Due to differences in species susceptibility, mercury must remain suspect as a causative agent for at least a small portion of the lowered reproductive success in the brown pelican in South Carolina. The herring gull appears to be much more resistant to DDE-induced eggshell thinning (36) than the brown pelican. Perhaps the case against mercury weakens when one considers that 16 of the 49 eggs from Florida contained 0.5 $\mu\text{g/g}$ or more mercury, and one on the verge of hatching contained 1.43 $\mu\text{g/g}$ (Table 1). The pelican population in Florida has been considered stable over the past five years, and reproductive success is considered normal (54). An occasional egg from the Atlantic Coast of Florida contained levels of dieldrin or DDE that are thought to be deleterious. However, the bulk of the residues in all areas of Florida are low enough that one would not expect these residues to induce widespread, long-term, adverse effects on the populations there.

It is probable that several pollutants are involved in the population decline of brown pelicans in South Carolina. There is a possibility that other pollutants not identified in this study may also have played some role. Storms and high tides have temporarily disrupted nesting activities at times, but the effects of these factors is thought to be minimal because the brown pelican is known to be a persistent renester (55). Predation appears to be a minor factor in loss of eggs and young in South Carolina. The islands are isolated, and mammalian predators are not likely to gain access to them. If they did, they would not likely establish permanent residence; cover is sparse, and severe storms sometimes inundate the islands. There are no effective avian egg predators around the colonies except for the herring gull (*Larus argentatus*) and the ring-billed gull (*Larus delawarensis*). These are present in small numbers, and they have not been observed eating pelican eggs. Laughing gulls (*Larus atricilla*) nest in large numbers in each colony, but they apparently have little facility for preying on pelican eggs.

THE POPULATION DECLINE IN SOUTH CAROLINA

Historically, little is known of the numbers of brown pelicans occurring in the colonies in South Carolina; however, we have a fairly good record of the numbers associated with the colony on the Cape Romain National Wildlife Refuge since the 1930's. The pelicans on the Refuge have nested on Marsh Island (Vessel Reef), Bird Bank, White Banks, Cape Island, and Raccoon

Key (Sandy Point) at various times in the past; as many as three colony sites on the refuge have been occupied at one time (Table 11).

The breeding population on the Cape Romain National Wildlife Refuge has remained relatively stable through the years. Sprunt and Chamberlain (55) reported a maximum of 800 breeding pairs in 1946. However, E. Milby Burton (personal communication) counted over 1,000 nests in one colony on the refuge during the 1930's. The number of estimated nesting pairs on the Cape Romain Refuge ranged from an estimated 500 to 650 from 1962 through 1968; yet a thorough count in 1969 revealed over 1,000 nests.

From 1947 to the early 1960's, Deveau Bank was apparently the major colony; some interchange of breeding birds apparently occurred between this colony and the Cape Romain colony. There is still apparently a great deal of interchange between the two colonies, but Cape Romain is now the major colony. In 1969, the birds began to nest early on Deveau Bank, but they deserted in the nest-building phase (T. A. Beckett, III, personal communication), and most of these birds probably moved to Marsh Island. In 1970, nesting pairs increased on Deveau Bank, and although breeding pairs decreased in the Cape Romain colony, it still contained more breeding birds than Deveau Bank. With the apparent movement of breeding birds between the two colonies from year to year, population trends in South Carolina seem to be valid only if both colonies are considered together.

Several other islands have been used for nesting sites in South Carolina (Table 11). Existence of some of these colonies is known only from published oological records (1) and from data taken from eggs in the Charleston Museum. The record of the pelican colony at the mouth of the Stono River is based on circumstantial evidence, but the island is still in existence and is considered suitable for brown pelican nesting. The exact location of the Bay Point colony near Beaufort, where eggs were collected in 1901, is not known because there are two locations called Bay Point, one on either side of Beaufort. It is not known whether a suitable nesting island is present at either of these locations. Thus pelicans have nested on at least 11 sites in South Carolina. There are relatively few islands in South Carolina that are acceptable to the brown pelican as nesting sites. Two principal requirements of pelican nesting islands seem to be isolation from mammalian predators, particularly raccoons (*Procyon lotor*), and sufficient elevation of the islands to prevent widespread flooding of nests. Of the two islands currently used for nesting, the pelicans have nested on Marsh Island continuously since 1967 and on Deveau Bank continuously since about 1946. There are no known nesting records for pelicans on Deveau Bank until 1946; the Bank was used as a bombing range

in World War II. Marsh Island has been used for nesting at various times since at least 1901. There is some evidence that two colonies of pelicans existed near Beaufort in 1943. A collector (E. J. DeCamps) took eggs from both colonies; he listed one nesting island as Bird Bank, St. Helena Sound (now known as Egg Bank), but he did not list a name for the other island, indicating only that it was 18 miles east of Beaufort. Egg Bank is 12 miles east of Beaufort, while Bay Point (Edisto Island) is approximately 18 miles east of Beaufort. Thus it seems very likely that two colonies existed near Beaufort in 1943, and that pelicans from both colonies moved to Deveau Bank. Mason (26) described the colony on Egg Bank as small, but gave no population estimate.

It seems clear that the pelicans on Deveau Bank have experienced a drastic population decline beginning at least 10 years ago. With the large nesting population of over 5,000 nesting pairs, the population decline may have progressed for several years before it was noticed. By 1969, the population on Deveau Bank had dwindled to approximately 250 nesting pairs; the number increased to 479 pairs in 1970. My (L.J.B.) experience with the island since 1969 leads me to believe that space for at least 2,000 to 3,000 nests is currently available.

The most accurate data on the number of breeding birds and reproductive success in South Carolina were obtained in 1969 and 1970. Over this period the number of nests was actually counted except for an estimate for Deveau Bank in 1969. The number of young fledged was estimated for each colony. According to age-specific mortality data obtained from birds banded during the period 1946 to 1966 in North and South Carolina, approximately 1.2 to 1.5 young must be fledged per breeding female per year in order to maintain a stable population (56). Based on this estimate, the number of young fledged in South Carolina in 1969 and 1970 was insufficient to maintain a stable population. The level of eggshell thinning (17%) in South Carolina is within the 15 to 20% level of thinning that has been associated with population declines of certain birds when such thinning occurs consistently over a period of years (27). The decline is greater than that recorded in black ducks and mallards fed DDE; these birds have also experienced lowered reproductive success (28, 29).

Conclusion

DDE is the agent responsible for all or most of the eggshell thinning of brown pelican eggs observed in all areas where eggs were collected. DDE, because of its propensity for inducing deleterious effects in experimental birds, is suspected of being the principal agent in the lowered reproductive success and resultant population declines in South Carolina and California pelicans.

However, other pollutants, particularly dieldrin, may adversely affect reproductive success in ways unrelated to shell thinning.

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TABLE 1.—Residues of environmental pollutants in brown pelican eggs

COLONY	$\mu\text{G/G}$ (FRESH WET WEIGHT)							
	<i>p,p'</i> -DDE	<i>p,p'</i> -DDD	<i>p,p'</i> -DDT	DIELDRIN	PCB'S	MERCURY	HE ¹	LEAD
SOUTH CAROLINA, 1969								
Cape Romain	4.22	0.75	0.77	0.78	5.4	0.65	ND ²	Tr ³
	3.64	0.91	0.99	0.60	3.9	0.34	ND	Tr
	5.38	1.79	1.70	1.10	3.8	0.12	ND	Tr
	3.25	1.36	0.83	0.78	4.8	0.29	ND	Tr
	6.55	1.62	1.45	1.63	5.5	0.31	ND	Tr
	4.42	0.94	0.99	1.37	5.7	0.34	ND	Tr
G.M. ⁴ Median	4.45	1.17	1.08	0.98	4.79	0.31	—	—
	4.32	1.15	0.99	0.94	5.10	0.33	—	—
Deveaux Bank	10.60	2.88	1.86	1.31	11.0	0.26	ND	Tr
	8.70	1.63	ND	0.70	6.6	0.18	ND	Tr
	4.55	1.59	ND	0.60	10.4	0.28	ND	Tr
	6.32	2.21	ND	1.25	5.8	0.26	Tr	Tr
	3.93	1.19	Tr	0.87	3.8	0.25	ND	Tr
	6.36	1.81	—	0.90	6.99	0.24	—	—
G.M. Median	6.32	1.63	—	0.87	6.60	0.26	—	—
SOUTH CAROLINA, 1970								
Cape Romain	0.80	0.20	0.20	0.20	2.0	0.90	ND	NR ⁵
	3.30	0.70	0.90	0.80	4.0	0.50	Tr	NR
	2.00	0.80	0.70	0.60	4.0	0.40	ND	NR
	2.30	0.60	0.50	0.50	3.0	0.40	ND	NR
	8.80	0.80	0.70	1.30	6.0	0.30	Tr	NR
	7.80	1.30	0.80	1.30	8.0	0.30	Tr	NR
	3.00	0.80	0.60	1.00	6.0	0.60	Tr	NR
	2.10	0.40	0.50	0.40	4.0	0.50	ND	NR
	1.70	0.50	0.50	0.30	4.0	0.50	ND	NR
	3.70	1.40	0.80	0.90	8.0	0.30	Tr	NR
	2.83	0.66	0.58	0.62	4.52	0.44	—	—
	2.65	0.75	0.65	0.70	4.00	0.45	—	—
G.M. Median								

TABLE 1.—Residues of environmental pollutants in brown pelican eggs—Continued

COLONY	$\mu\text{G/G}$ (FRESH WET WEIGHT)							
	<i>p,p'</i> -DDE	<i>p,p'</i> -DDD	<i>p,p'</i> -DDT	Dieldrin	PCB's	MERCURY	HE ¹	LEAD
FLORIDA ATLANTIC COAST, 1969								
Port Orange	1.72	0.24	0.45	ND	1.7	0.48	ND	Tr
	0.16	0.34	0.12	ND	6.9	0.35	ND	Tr
	5.93	1.74	Tr	0.39	7.1	0.23	ND	Tr
	2.36	1.53	ND	0.66	2.7	0.37	ND	Tr
	1.77	1.07	0.41	0.75	5.3	0.12	ND	Tr
G.M. Median	1.47	0.75	0.14	0.22	4.12	0.28	—	—
	1.77	1.07	0.12	0.39	5.30	0.35	—	—
Cocoa Beach	4.03	1.18	0.88	1.52	7.5	0.20	0.10	Tr
	2.37	0.80	0.56	0.39	2.4	0.44	ND	Tr
	3.49	0.81	0.90	0.67	4.6	0.36	ND	Tr
	3.07	0.92	0.67	0.89	7.4	0.29	Tr	Tr
	0.92	0.18	0.42	0.29	1.3	1.48	ND	Tr
G.M. Median	2.48	0.66	0.66	0.63	3.80	0.42	—	—
	3.07	0.81	0.67	0.67	4.60	0.36	—	—
Pelican Island	1.14	1.99	0.66	0.55	1.3	0.39	0.29	Tr
	2.28	1.75	0.56	0.57	2.8	0.22	ND	Tr
	1.06	0.19	0.30	0.10	1.4	0.41	ND	Tr
	2.48	0.67	0.99	0.32	2.4	0.36	Tr	Tr
	6.01	1.98	0.22	1.09	5.5	0.34	Tr	Tr
G.M. Median	2.10	1.00	0.47	0.41	2.32	0.34	—	—
	2.28	1.75	0.56	0.55	2.40	0.36	—	—
Fort Pierce	2.81	1.09	0.67	0.57	3.9	0.48	ND	Tr
	2.11	0.83	0.30	0.54	1.9	0.68	ND	Tr
	1.54	0.72	0.38	0.20	1.4	0.70	ND	Tr
	3.39	0.97	0.67	0.28	2.4	0.52	ND	Tr
	3.15	1.01	0.66	0.25	2.5	0.57	ND	Tr
	3.00	1.23	0.67	0.31	1.9	0.16	ND	Tr
	1.74	0.83	0.56	0.26	1.0	0.38	ND	Tr
	2.44	0.94	0.54	0.32	1.98	0.46	—	—
G.M. Median	2.81	0.97	0.66	0.26	1.90	0.52	—	—
FLORIDA GULF COAST, 1969								
Cedar Key	0.98	0.31	0.20	Tr	Tr	0.47	ND	Tr
	0.85	0.31	0.14	Tr	Tr	0.16	ND	Tr
	0.84	0.41	0.22	0.15	Tr	0.18	ND	Tr
	5.54	0.96	0.39	Tr	4.2	0.35	ND	Tr
	1.65	0.59	0.30	0.12	1.4	0.31	ND	Tr
	1.00	0.20	0.27	Tr	Tr	0.48	ND	Tr
	1.32	0.23	0.26	Tr	1.2	0.49	ND	Tr
	1.36	0.37	0.24	0.08	0.89	0.32	—	—
G.M. Median	1.00	0.31	0.26	0.05	0.50	0.35	—	—
	1.00	0.31	0.26	0.05	0.50	0.35	—	—
Pinellas	1.23	0.63	0.44	0.13	1.6	0.40	Tr	Tr
	1.72	0.65	0.52	0.26	1.8	0.17	Tr	Tr
	2.44	0.99	0.57	0.27	1.5	0.41	Tr	Tr
	3.78	1.95	ND	0.40	2.9	1.21	Tr	Tr
	1.42	0.62	0.54	Tr	1.1	0.66	ND	Tr
G.M. Median	1.94	0.87	0.32	0.18	1.69	0.47	—	—
	1.72	0.65	0.52	0.26	1.60	0.41	—	—
Boca Grande Pass	1.18	0.29	ND	0.11	Tr	0.43	ND	Tr
	0.46	0.12	0.11	Tr	Tr	0.16	ND	Tr
	0.78	0.23	ND	Tr	Tr	0.42	ND	Tr
	0.70	0.36	0.24	0.21	Tr	0.45	ND	Tr
	0.52	0.15	0.22	Tr	Tr	0.40	ND	Tr
G.M. Median	0.69	0.22	0.11	0.09	0.50	0.35	—	—
	0.70	0.23	0.11	0.05	0.50	0.42	—	—
Hemp Island	1.76	0.37	0.33	0.17	2.0	0.24	ND	Tr
	1.87	0.45	0.52	Tr	Tr	0.59	ND	Tr
	1.86	0.65	0.52	0.15	Tr	0.78	ND	Tr
	1.19	0.24	0.19	Tr	Tr	0.62	ND	Tr
	1.66	0.52	0.32	0.11	Tr	0.86	ND	Tr
G.M. Median	1.65	0.42	0.35	0.09	0.66	0.57	—	—
	1.76	0.45	0.33	0.11	0.50	0.62	—	—
Matlacha	1.32	0.19	0.22	Tr	1.0	0.75	ND	Tr
	3.01	1.51	0.71	Tr	2.3	1.47	Tr	Tr
	1.44	1.02	0.39	0.22	1.4	0.86	ND	Tr
	2.17	0.70	0.39	0.10	1.0	0.82	ND	Tr
	0.90	0.28	0.21	Tr	Tr	0.50	ND	Tr
G.M. Median	1.62	0.56	0.35	0.08	1.10	0.83	—	—
	1.44	0.70	0.39	0.05	1.00	0.82	—	—

TABLE 1.—Residues of environmental pollutants in brown pelican eggs—Continued

COLONY	$\mu\text{G/G}$ (FRESH WET WEIGHT)							
	<i>p,p'</i> -DDE	<i>p,p'</i> -DDD	<i>p,p'</i> -DDT	DIELDRIN	PCB's	MERCURY	HE ¹	LEAD
FLORIDA, FLORIDA BAY, 1970								
Nest Key	0.81	ND	ND	ND	1.1	0.98	ND	Tr
	0.65	0.16	ND	ND	Tr	0.29	ND	Tr
	1.59	0.34	0.17	0.28	2.5	0.78	ND	Tr
	0.90	0.12	ND	ND	0.6	0.38	ND	Tr
	1.72	0.36	0.16	Tr	1.4	0.84	ND	Tr
G.M. Median	1.05	0.16	—	—	0.9	0.59	—	—
	0.90	0.16	—	—	1.1	0.78	—	—
Buchanan Key	2.87	0.50	0.35	0.33	2.7	0.94	ND	Tr
	0.62	0.13	ND	ND	Tr	0.59	ND	Tr
	1.66	0.10	ND	0.15	1.8	0.86	ND	Tr
	0.51	0.18	ND	ND	Tr	0.49	ND	Tr
	1.15	0.20	0.13	0.14	1.3	0.94	ND	Tr
G.M. Median	1.11	0.19	—	—	0.8	0.74	—	—
	1.15	0.18	—	—	1.3	0.86	—	—
Fanny Key	0.56	0.15	ND	ND	Tr	0.51	ND	Tr
	0.68	0.10	ND	ND	Tr	0.47	ND	Tr
	0.96	0.21	ND	ND	0.6	0.09	ND	Tr
	0.40	Tr	ND	ND	Tr	0.57	ND	Tr
	1.64	0.23	0.24	ND	2.1	0.11	ND	Tr
G.M. Median	0.75	0.13	—	—	0.5	0.27	—	—
	0.68	0.15	—	—	Tr	0.47	—	—
Marquesas Key	1.07	0.12	0.15	ND	Tr	0.35	ND	Tr
	0.82	ND	0.11	ND	Tr	0.45	ND	Tr
	1.46	0.14	0.25	ND	Tr	0.53	ND	Tr
	1.20	0.14	0.12	ND	Tr	0.45	ND	Tr
	1.45	0.14	0.16	ND	1.1	0.54	ND	Tr
G.M. Median	1.17	0.11	0.15	—	0.3	0.46	—	—
	1.20	0.14	0.15	—	Tr	0.45	—	—
CALIFORNIA, 1969								
Anacapa ⁶	76.1	1.3	1.9	0.2	8.0	0.42	ND	Tr
	50.6	1.4	1.9	0.1	5.0	0.36	ND	Tr
	135.0	2.4	3.8	0.2	12.0	0.31	ND	Tr
	60.2	0.3	0.6	Tr	3.2	0.17	ND	Tr
	39.5	0.5	1.2	Tr	Tr	0.40	ND	Tr
	97.8	3.4	4.6	0.1	8.0	0.30	ND	Tr
	63.2	1.3	1.6	Tr	5.0	0.56	ND	Tr
	65.0	0.9	1.5	Tr	Tr	0.34	ND	0.17
	70.0	1.3	1.4	Tr	6.0	0.31	ND	Tr
	98.4	1.5	1.9	0.2	4.0	0.33	ND	Tr
	71.35	1.18	1.76	0.09	3.60	0.34	—	0.06
	67.50	1.30	1.90	0.08	5.00	0.34	—	0.05
G.M. Median								

¹ HE = Heptachlor epoxide.² ND = No residue detected.³ Tr = Trace of residue; a trace is considered $<1.0 \mu\text{g/g}$ for PCB's and $<0.1 \mu\text{g/g}$ for all other residues.⁴ G.M. = Geometric Mean.⁵ NR = Analysis not run.⁶ Each of the 10 eggs from California contained from trace to $0.2 \mu\text{g/g}$ of both *o,p'*-DDT and *o,p'*-DDD.

TABLE 2.—Comparisons of brown pelican egg residues by geographic areas, 1969

AREA	NUMBER OF EGGS	GEOMETRIC MEAN ($\mu\text{G/G}$) ¹					
		<i>p,p'</i> -DDE	<i>p,p'</i> -DDD	<i>p,p'</i> -DDT	DIELDRIN	PCB's	MERCURY
Florida Gulf Coast	27	1.37 A ² (1.09-1.72)	0.43 A (0.33-0.57)	0.25 A (0.19-0.34)	0.10 A (0.07-0.12)	0.89 A (0.68-1.16)	0.46 A (0.36-0.57)
Florida Atlantic Coast	22	2.24 B (1.60-3.13)	0.83 B (0.61-1.14)	0.40 A (0.27-0.60)	0.36 B (0.24-0.53)	2.81 B (2.11-3.74)	0.37 AB (0.29-0.48)
South Carolina	11	5.24 C (4.08-6.72)	1.43 C (1.09-1.87)	0.37 A (0.13-1.09)	0.94 C (0.77-1.16)	5.68 C (4.45-7.26)	0.28 B (0.21-0.36)
California	10	71.70 D (55.38-91.93)	1.18 BC (0.71-1.94)	1.76 B (1.17-2.65)	0.09 A (0.06-0.14)	3.60 BC (1.63-7.95)	0.42 AB (0.24-0.71)

¹ 95% confidence limits in parentheses.² A significant difference ($P<0.05$) among means of a chemical is indicated for those residues not sharing a common letter. Means were separated using Duncan's New Multiple Range test with Kramer's extension for unequal replication. (Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics, 12: 307-310.)

TABLE 3.—Association of egg residues with one another, egg basis

CHEMICAL	SIMPLE CORRELATION COEFFICIENT				
	DDD	DDT	DIELDRIN	PCB's	MERCURY
CALIFORNIA					
DDE	0.698 ¹	0.679 ¹	0.688 ¹	0.655 ¹	—0.153
DDD	—	0.950 ²	0.577	0.620	0.350
DDT	—	—	0.609	0.500	0.310
Dieldrin	—	—	—	0.583	0.076
PCB's	—	—	—	—	—0.050
SOUTH CAROLINA					
DDE	0.835 ²	0.038	0.845 ²	0.751 ²	—0.698 ²
DDD	—	—0.037	0.767 ²	0.732 ²	—0.803 ²
DDT	—	—	0.259	—0.010	0.100
Dieldrin	—	—	—	0.618 ²	—0.555 ²
PCB's	—	—	—	—	—0.401
FLORIDA					
DDE	0.732 ²	0.314 ¹	0.531 ²	0.559 ²	0.032
DDD	—	0.272	0.647 ²	0.632 ²	—0.025
DDT	—	—	0.262	0.239	0.033
Dieldrin	—	—	—	0.610 ²	—0.245
PCB's	—	—	—	—	—0.217

¹ P<0.05² P<0.01

TABLE 4.—Residues of environmental pollutants in an adult male bown pelican that died in tremors

CHEMICAL	μG/G (WET WEIGHT)	
	CARCASS	BRAIN
p,p'-DDE	11.04	11.30
p,p'-DDD	2.80	2.17
p,p'-DDT	0.31	1.26
Dieldrin	1.24	1.82
HE	Tr	Tr
PCB's	20.0	10.0
Mirex	NR	NR
Mercury	1.57	NR

TABLE 5.—Residues of environmental pollutants in carcasses of brown pelicans

APPROXIMATE AGE	SEX	μG/G (WET WEIGHT)					
		p,p'-DDE	p,p'-DDD	p,p'-DDT	DIELDRIN	PCB's	MIREX
SOUTH CAROLINA							
1 week	unk ¹	0.79	ND	ND	0.19	1.3	ND
6 weeks	♀	0.20	ND	ND	ND	Tr	ND
6 weeks	unk	0.29	ND	ND	ND	Tr	ND
Immature	♀	9.20	0.74	0.12	1.50	19.0	0.22
Adult	♂	25.20	0.84	0.38	0.84	35.0	1.06
FLORIDA: ATLANTIC COAST							
4 weeks	♀	0.10	ND	ND	ND	Tr	ND
10 weeks	unk	0.37	1.21	0.51	0.44	7.5	ND
13 weeks	♂	1.35	0.54	ND	0.34	3.3	ND
Immature	♀	15.00	1.90	0.69	1.60	29.0	0.20
Adult	♂	8.00	0.76	ND	0.61	12.0	0.20
FLORIDA: FLORIDA BAY							
3 weeks	unk	0.34	ND	ND	ND	Tr	ND
7 weeks	♀	0.29	ND	ND	ND	Tr	ND
Immature	♂	1.20	0.46	0.12	0.13	1.0	ND
Adult	♂	3.65	0.70	0.38	0.47	4.4	ND
Adult	♀	1.20	0.38	ND	ND	3.0	ND
FLORIDA: GULF COAST							
1 week	unk	0.13	ND	ND	ND	Tr	ND
4 weeks	unk	0.26	0.10	0.79	ND	1.2	ND
14 weeks	♀	1.00	1.10	0.17	0.29	3.5	0.17
Immature ²	♂	4.55	0.84	ND	0.60	19.0	0.31
Immature	♂	31.00	1.90	1.20	1.80	34.0	0.30
Adult	♀	9.60	0.63	ND	0.74	19.5	1.45

¹ Sex not determined.² Sick bird.

TABLE 6.—Brown pelican pre-1947 eggshell thickness by decades, Florida

DECADE	NUMBER OF EGGS	THICKNESS, MM
		MEAN $\pm$ STANDARD ERROR
1890-1899	44	0.560 $\pm$ 0.006 ¹
1900-1909	12	0.566 $\pm$ 0.008
1910-1919	33	0.568 $\pm$ 0.007
1920-1929	39	0.549 $\pm$ 0.005
1930-1939	38	0.553 $\pm$ 0.006

¹ Means not significantly different ($P>0.05$) as tested using analysis of variance. Eggs from 1880-1889 omitted due to small sample size.

TABLE 9.—Association of eggshell measurement parameters with one another, egg basis

PARAMETER	SIMPLE CORRELATION COEFFICIENT ¹		
	WEIGHT	THICKNESS INDEX	PERCENT OF PRE-1947 THICKNESS
FLORIDA, SOUTH CAROLINA, AND CALIFORNIA			
Thickness	0.832	0.893	0.998
Weight	—	0.849	0.831
Thickness Index	—	—	0.903

¹ $P<0.01$

TABLE 7.—Thicknesses of brown pelican eggshells from 17 colonies, 1970

COLONY	NUMBER OF EGGS	MEAN THICKNESS ¹ , MM
SOUTH CAROLINA		
Deveaux Bank	20	0.460 $\pm$ 0.011 A ²
Cape Romain (Marsh Island)	18	0.461 $\pm$ 0.009 A
FLORIDA		
Cocoa Beach (Hall Island)	10	0.482 $\pm$ 0.019 AB
Pinellas (Tarpon Key)	10	0.487 $\pm$ 0.015 ABC
Crane Island	10	0.491 $\pm$ 0.009 ABC
Port Orange	9	0.497 $\pm$ 0.009 ABCD
Pelican Island	9	0.498 $\pm$ 0.017 ABCD
Cortez	10	0.502 $\pm$ 0.012 BCD
Matlacha Pass	10	0.504 $\pm$ 0.019 BCD
Fort Pierce	9	0.504 $\pm$ 0.009 BCD
Boca Grande Pass (Bird Key)	10	0.517 $\pm$ 0.014 BCD
Hemp Island	10	0.519 $\pm$ 0.015 BCD
Fanny Key	7	0.523 $\pm$ 0.019 BCD
Cedar Key (Seahorse Key)	10	0.531 $\pm$ 0.016 CD
Nest Key	10	0.532 $\pm$ 0.012 CD
Marquesas Key	10	0.541 $\pm$ 0.012 D
Buchanan Key	10	0.545 $\pm$ 0.013 D

¹ Mean $\pm$ standard error.

² A significant difference ($P<0.05$) among thickness means is indicated for those means not sharing a common letter. Means were separated using Duncan's New Multiple Range Test with Kramer's extension for unequal replication. (See footnote 2, Table 2.)

TABLE 10.—Reproductive success of brown pelicans in South Carolina, 1969-70

YEAR	COLONY	NUMBER OF NESTS	NUMBER OF YOUNG FLEDGED	YOUNG FLEDGED PER NEST
1969	Cape Romain	1016	900	0.82
	Deveaux Bank	250	80	0.32
	Totals or mean	1266	980	0.78
1970	Cape Romain	637	500	0.78
	Deveaux Bank	479	445	0.93
	Totals or mean	1116	945	0.85

TABLE 8.—Thicknesses of brown pelican eggshells, comparisons of pre-1947, 1969, and 1970 measurements

MEAN THICKNESS $\pm$ STANDARD ERROR		
PRE-1947 ¹	1969	1970
SOUTH CAROLINA		
0.557 $\pm$ 0.010 (23) A ²	0.463 $\pm$ 0.006 (49) B	0.461 $\pm$ 0.007 (38) B
FLORIDA		
0.557 $\pm$ 0.003 (172) A	0.515 $\pm$ 0.005 (81) B	0.512 $\pm$ 0.004 (144) B

¹ Sample size in parentheses.

² A significant difference ($P<0.05$) among thickness means for each state is indicated for those means not sharing a common letter. Means were separated using Duncan's New Multiple Range Test with Kramer's extension for unequal replication. (See footnote 2, Table 2.)

TABLE 11.—Islands used for nesting by the brown pelican in South Carolina

ISLAND	YEAR
Unidentified, Georgetown County	1934 ¹
Raccoon Key (Sandy Point)	1931 ² ; 1943-1946; 1952 ³
Cape Island	1939-1942 ³
White Banks	1956; 1958-1960; 1963; 1965 ³
Marsh Island (Vessel Reef)	1947-1948; 1951; 1955-1959; 1962; 1964-1965; 1967 through 1972 ³
Bird Bank	1901; 1915 ¹ ; 1926 ⁴ ; 1928 ² ; 1951; 1957-1961; 1963; 1965-1966 ³
Unidentified, on beach near Charleston	1901 ¹
Bird Key (mouth of Stono River)	Unknown ⁵
Deveaux Bank	1947 through 1972 ⁶
Egg Bank (near Beaufort, also called Bird Bank)	1904 ⁴ ; 1943 ¹
Unidentified, 18 mi. east of Beaufort	1943 ¹
Bay Point (large colony)	1901 ¹

¹ Anderson and Hickey (see LITERATURE CITED, reference 1).

² E. Milby Burton (personal communication).

³ Records of the Cape Romain National Wildlife Refuge. I (L.J.B.) have visited the Marsh Island colony each year since 1969.

⁴ Data taken from egg collection in Charleston Museum.

⁵ Robert Gracy (personal communication: people associated with this area informed him that pelicans once nested here).

⁶ Beckett (see LITERATURE CITED, reference 2). I (L.J.B.) visited this colony each year since 1969.

Residues of Organochlorine Pesticides, Mercury, and PCB's in Mourning Doves from Eastern United States—1970-71

J. F. Kreitzer¹

ABSTRACT

Mourning dove (*Zenaidura macroura*) breast muscle samples from birds collected in 1970-71 from Rhode Island, Pennsylvania, Maryland, Missouri, Kentucky, Virginia, Arkansas, Tennessee, North Carolina, South Carolina, Mississippi, Alabama, Georgia, Louisiana, and Florida were found to contain residues of DDT, DDE, DDD, polychlorinated biphenyls, dieldrin, mirex, mercury, and heptachlor epoxide in amounts not considered hazardous to consumers, human or nonhuman. There were 145 birds involved, 7 to 10 from each State. Residues of DDT plus DDE plus DDD averaged 5.83 ppm lipid weight (0.068 ppm wet weight); those of PCB's, 9.75 ppm lipid weight (0.121 ppm wet weight); compounds were found in all birds. Heptachlor epoxide and dieldrin were found in little more than trace amounts: heptachlor in all birds, dieldrin in 73. Mirex was found only in birds from South Carolina, Georgia, and Florida, averaging 4.32 ppm lipid weight (0.046 ppm wet weight) in eight birds. Less than 0.05 ppm (wet weight) mercury was found in all birds except 10, in which it ranged from 0.07 to 0.67 ppm. The 10 were from Rhode Island, Pennsylvania, Maryland, Virginia, Tennessee, and Alabama.

Introduction

This paper reports the residues of organochlorine pesticides, polychlorinated biphenyls, and mercury in mourning doves (*Zenaidura macroura*) of the eastern United States. The study was undertaken to determine whether the small but steady decline of mourning dove populations in the United States during 1960-1970 (Migratory Bird Populations Station, unpublished data) might be related to environmental contaminants, and whether the level of these contaminants in the tissues need cause concern for their consumption as human food.

Certain game birds have been found to contain undesirably high levels of toxicants. For example, 28 of 32 pheasants (*Phasianus colchicus*) found dead or dying in Sweden, 1957-1963, contained between 25 and 140 ppm mercury in liver and kidney tissues, and 16 of 27 pheasants shot or trapped during the same period contained more than 1 ppm (range: 1-20 ppm) (1).

In 1970, the Canadian government closed the hunting season for woodcock (*Philohela minor*) in New Brunswick because of an average level of 56 ppm DDT (lipid basis) in representative samples taken throughout the Province. In 1969, the Province of Alberta closed the seasons for pheasant (*Phasianus colchicus*) and Hungarian partridge (*Perdix perdix*) because of high levels of mercury in the liver; residues averaged 2.8 ppm in pheasants and 1.1 ppm in partridges (2). Although the season was not closed for sharp-tailed grouse (*Pedioecetes phasianellus*), concentrations of mercury in the liver were as high as 1.1 ppm.

In western Montana 73 samples of fatty tissues from 69 blue grouse (*Dendragapus obscurus*), collected ½ to 685 days after their range had been sprayed with DDT at ½ lb/acre, contained DDT-derived residues ranging from 0.9 to 280 ppm (3). In this instance the U.S. DHEW Food and Drug Administration recommended that persons eating game from pesticide-treated areas be warned to trim away fat to the extent practical and thereby reduce exposure to such residues.

Materials and Methods

Birds were collected from all States east of the Mississippi that permit the hunting of mourning doves, except for Illinois, Delaware, and West Virginia. Eastern samples came from Rhode Island, Pennsylvania, Mary-

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land, Kentucky, Tennessee, Virginia, North Carolina, South Carolina, Mississippi, Alabama, Georgia, and Florida. Three dove-hunting states west of the Mississippi were also included: Missouri, Arkansas, and Louisiana. Ten birds were taken from each State except for Arkansas and Virginia which produced nine each, and Pennsylvania, which produced seven. Thus 145 birds were available for tissue analysis.

Carcasses were frozen at the time of collection. At preparation of the samples they were partly thawed and a 10-g sample of breast muscle was taken from each. Samples were placed in a glass jar that had been cleaned with nitric acid, acetone, and hexane; then they were refrozen.

Analyses were made at WARF Institute, Inc., Madison, Wisconsin, by the following methods:

For organochlorines and PCB's, samples were weighed, dried at 40°C for 72-96 hours, reweighed, then ground with sodium sulfate and extracted with petroleum ether:ethyl ether (170 ml:70 ml) on a Soxhlet extractor for 8 hours. They were cleaned and separated into two fractions by passage through a Florisil Column (petroleum ether:ethyl ether, 95:5, 85:15). An aliquot of the sample was passed through a standardized silicic acid-celite column, with typical elutions of 260 ml petroleum ether:210 ml 1:19:80 acetonitrile:hexane:dichloromethane. Analysis was by a Barber-Coleman Pesticide Analyzer Model 5360. The column was glass, 4 ft by 4 mm, packed with 5% DC-200°C (column), and 240°C (detector); carrier gas was nitrogen at a flow rate that eluted *p,p'*-DDT in 6-8 minutes. Lipid weight was determined from an aliquot of the extract which was reduced to dryness on a stream bath and placed in a 40°C oven for 2 to 4 hours before weighing. Three-column confirmations of compounds found were made on a random selection of tissue samples.

Total mercury was determined by cold vapor atomic absorption. A 10-g portion was digested by refluxing with sulfuric nitric acid mixture (4). A mixture of hydroxylamine, stannous chloride, and sulfuric acid was added to the digest to reduce the mercury (II) ions to mercury metal. The sample was aerated (3 liters/min) and passed through the absorption cell.

Recoveries of organochlorine pesticides from spiked samples were 75-105%; those of PCB's (based on Aroclor 1254) were 60-85%; and those of mercury were 95%. Residues were not corrected for recovery. The limit of sensitivity was from 0.001 to 0.006 ppm for organochlorines, and 0.05 ppm for mercury.

Analyses of variance were made using log transformations.

Results and Discussion

Results of analyses are in Tables 1 and 1A. Means given in the tables and in the text are arithmetic. Locations of collection sites with corresponding concentrations of DDE and PCB in the birds are shown in Figures 1 and 2.

Because of the low residues in many samples and the numerous "less than" values, overall means were computed only for DDT and its metabolites (DDE and DDD), mirex, and PCB's. Means were: 5.83 ppm lipid weight (0.068 ppm wet weight) DDT (including metabolites); 4.32 ppm lipid weight (0.046 ppm wet weight) mirex; and 9.75 ppm lipid weight (0.121 ppm wet weight) PCB's. Statistical comparisons were possible only for DDE and PCB's (Table 2). These comparisons are strictly between collecting sites, not between states: the sites were not selected at random. There were no clearcut differences between northern sites and southern sites or between other geographical regions.

Mercury was found in small amounts, less than 0.05 ppm (wet weight) except in 10 birds in which it ranged from 0.07 to 0.67 ppm. The 10 were from Pennsylvania, Rhode Island, Maryland, Alabama, Tennessee and Virginia. Heptachlor epoxide and dieldrin were found in little more than trace amounts in most birds; only a few birds contained more than 1 ppm. Amounts of heptachlor epoxide ranged from 0.14 to 8.70 ppm lipid weight (<0.006 to 0.170 ppm wet weight), and those of dieldrin, from 0.11 to 10.22 ppm lipid weight (0.001 to 0.160 ppm wet weight).

DDT and its metabolites, PCB's, heptachlor epoxide, and mercury were found in all birds; dieldrin was found in 73; and mirex in 8.

The relatively small amounts of these compounds in the birds suggest there is little hazard, if any, to the birds themselves, or to the consumers, human or nonhuman, of the birds flesh. The amount of DDT and its metabolites does approach the amount of DDT permitted in ground beef by Federal regulations (7 ppm in the fat). But since dove meat has so little fat (1.3% in the tissues analyzed), an individual would have to eat nearly 26 pounds of it to ingest the amount of the pesticide permitted in 1 pound of hamburger. About 550 doves would be required.

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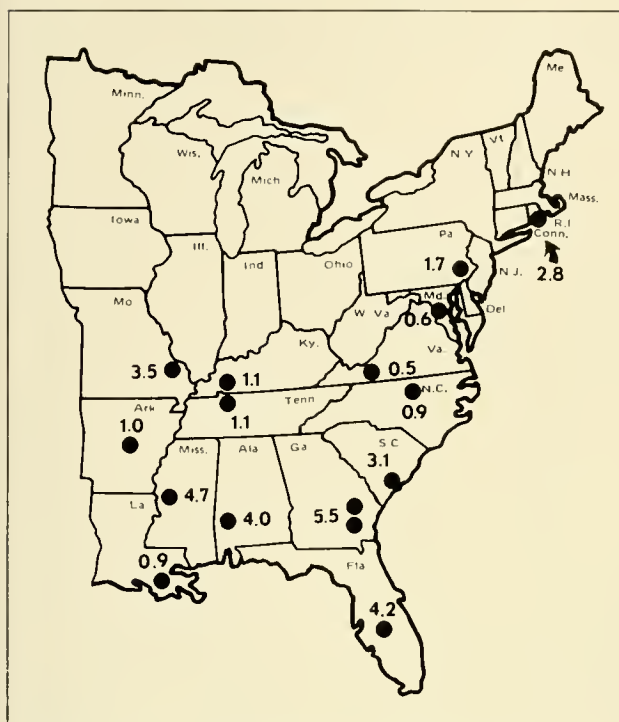


FIGURE 1.—Mean (geometric) concentration of DDE (lipid basis) in breast muscle of mourning doves, 1970-71¹

¹ Dots show approximate sites of collection.



FIGURE 2.—Mean (geometric) concentration of PCB (lipid basis) in breast muscle of mourning doves, 1970-71¹

¹ Dots show approximate sites of collection.

TABLE 1.—Residues of DDE, DDD, and DDT in breast muscle of mourning doves from 15 eastern hunting states, 1970-71

COLLECTION SITE	No. OF BIRDS	WT. BASIS	RESIDUES, PPM								
			DDE			DDD			DDT		
			LOW	HIGH	MEAN	LOW	HIGH	MEAN	LOW	HIGH	MEAN
Rhode Island	10	W	0.010	0.160	0.050	<0.006	0.034	0.008	<0.006	0.008	0.006
(Washington Co.)		L	0.550	9.898	3.072	<0.168	2.774	0.522	<0.144	0.561	0.351
Pennsylvania	7	W	0.006	0.440	0.084	<0.006	0.009	0.006	<0.006	0.011	0.006
(Berks Co.)		L	0.411	38.52	6.002	<0.223	0.632	0.412	<0.223	0.632	0.439
Maryland	10	W	<0.005	0.047	0.014	—	—	<0.006*	—	—	<0.006*
(Anne Arundel Co.) (Prince Georges Co.)		L	<0.175	4.338	0.962	<0.175	<0.938	—	<0.175	0.938	—
Missouri	10	W	0.021	0.220	0.073	<0.006	0.034	0.013	<0.006	0.064	0.013
(Cape Girardeau Co.)		L	2.555	10.44	4.984	<0.361	2.446	0.865	<0.400	3.218	0.871
Kentucky	10	W	<0.006	0.024	0.013	—	—	<0.006*	—	—	<0.006*
(Trigg Co.)		L	0.366	1.275	0.864	<0.235	<0.463	—	<0.235	<0.463	—
Virginia	9	W	<0.006	0.071	0.015	—	—	<0.006*	—	—	<0.006*
(Montgomery Co.)		L	0.273	7.350	1.466	0.273	<0.674	—	<0.273	<0.899	—

TABLE 1.—Residues of DDE, DDD, and DDT in breast muscle of mourning doves from 15 eastern hunting states, 1970-71
—Continued

COLLECTION SITE	No. OF BIRDS	WT. BASIS	RESIDUES, PPM								
			DDE			DDD			DDT		
			LOW	HIGH	MEAN	LOW	HIGH	MEAN	LOW	HIGH	MEAN
Arkansas	9	W	0.006	0.065	0.019	—	—	<0.006*	—	—	<0.006*
(Conway Co.)		L	0.541	6.140	1.730	<0.226	<0.682	—	<0.226	<0.682	—
Tennessee	10	W	0.005	0.085	0.023	<0.006	0.008	—	<0.006	0.012	—
(Stewart Co.)		L	0.417	8.468	1.777	<0.254	0.811	—	<0.254	1.261	—
North Carolina	10	W	0.006	0.039	0.014	—	—	<0.006*	—	—	<0.006*
(Wake Co.)		L	0.471	2.953	1.088	<0.258	<0.531	—	<0.258	<0.531	—
South Carolina	10	W	0.009	0.580	0.026	<0.006	0.024	0.010	<0.006	0.018	0.008
(Charleston Co.)		L	0.806	76.118	7.739	<0.302	3.176	1.020	<0.352	2.471	0.754
Mississippi	10	W	0.009	0.270	0.087	<0.006	0.046	0.012	<0.006	0.063	0.013
(Madison Co.)		L	0.559	26.415	7.724	<0.373	4.528	1.132	<0.132	6.226	1.219
Alabama	10	W	0.007	0.810	0.126	<0.006	0.013	0.008	<0.006	0.044	0.014
(Chocktaw Co.) (Mobile Co.)		L	0.389	78.00	11.490	<0.333	1.181	0.628	<0.333	2.835	1.182
Georgia	10	W	0.008	0.400	0.122	<0.006	0.050	0.016	<0.006	0.008	0.006
(Oconee Co.) (Toombs Co.)		L	0.678	35.60	9.379	<0.339	3.670	1.231	<0.339	3.511	1.234
Louisiana	10	W	<0.003	0.013	0.008	—	<0.007	—	—	<0.007	—
(Terrebonne Co.)		L	<0.220	2.368	1.064	<0.330	<0.882	—	<0.330	<0.882	—
Florida	10	W	0.010	0.250	0.089	<0.006	0.032	0.008	<0.006	0.150	0.008
(Highland Co.)		L	0.991	25.69	7.756	<0.444	3.303	0.732	<0.380	1.193	0.663

W = wet weight; L = lipid weight.

* = All birds reported at this value.

ND = Not detected; value = 0 in computing means.

Numerals in parentheses indicate number of birds in which the compound was not detected.

< quantities taken at face value in computing means.

Means are arithmetic.

TABLE 1A.—Residues of PCB's, dieldrin, mirex, and heptachlor epoxide in breast muscle of mourning doves from 15 eastern hunting states, 1970-71

COLLECTION SITE	No. OF BIRDS	WT. BASIS	RESIDUES, PPM											
			PCB			DIELDRIN			MIREX			HEPTACHLOR EPOXIDE		
			LOW	HIGH	MEAN	LOW	HIGH	MEAN	LOW	HIGH	MEAN	LOW	HIGH	MEAN
Rhode Island	10	W	0.091	0.520	<0.229	<0.005	0.160	—				<0.006	0.170	—(5)
(Washington Co.)		L	8.867	43.307	14.131	<0.566	10.22	—		ND		<0.143	7.936	—(5)
Pennsylvania	7	W	0.072	0.140	0.102	<0.005	0.016	—				<0.006	0.021	—(2)
(Berks Co.)		L	3.234	9.895	7.027	<0.526	1.012	—		ND		<0.223	1.310	—(2)
Maryland	10	W	0.046	0.180	0.088	—	—	0.005*(8)				—	—	<0.006*
(Anne Arundel Co.) (Prince Georges Co.)		L	3.179	21.406	5.929	<0.298	<0.781	—		ND		<0.175	0.411	—
Missouri	10	W	0.093	0.280	0.153	0.005	0.077	0.016				<0.006	0.027	—(1)
(Cape Girardeau Co.)		L	4.760	31.609	13.320	0.361	2.299	1.083		ND		<0.361	3.103	—(1)
Kentucky	10	W	0.061	0.200	0.118							—	—	<0.006*
(Triggs Co.)		L	4.573	15.583	8.326		ND			ND		<0.235	<0.500	—
Virginia	9	W	0.110	0.240	0.164							—	—	<0.006*
(Montgomery Co.)		L	8.864	23.810	15.520		ND			ND		<0.272	<0.674	—

TABLE 1A.—Residues of PCB's, dieldrin, mirex, and heptachlor epoxide in breast muscle of mourning doves from 15 eastern hunting states, 1970-71—Continued

COLLECTION SITE	No. OF BIRDS	Wt. Basis	RESIDUES, PPM											
			PCB			DIELDRIN			MIREX			HEPTACHLOR EPOXIDE		
			LOW	HIGH	MEAN	LOW	HIGH	MEAN	LOW	HIGH	MEAN	LOW	HIGH	MEAN
Arkansas (Conway Co.)	9	W	0.073	0.170	0.089	—	0.006	—(8)		ND		<0.006	0.015	—
		L	3.860	16.367	7.201	—	0.526	—(8)				<0.382	1.404	—
Tennessee (Stewart Co.)	10	W	0.075	0.240	0.164	<0.005	0.006	—(5)		ND		—	—	<0.006*
		L	6.042	24.234	12.320	<0.212	0.631	—(5)				<0.254	<0.541	—
North Carolina (Wake Co.)	10	W	0.042	0.082	0.061	—	—	<0.005*(8)		ND		—	—	<0.006*
		L	3.100	6.351	4.654	<0.258	<0.462	—(8)				<0.257	<0.531	—
South Carolina (Charleston Co.)	10	W	0.086	0.160	0.121	<0.005	0.024	—(2)	0.005	0.039	0.007(8)	—	—	<0.006*
		L	5.324	18.229	12.004	<0.251	3.012	—(2)	0.602	4.234	2.680(8)	<0.302	<1.008	—
Mississippi (Madison Co.)	10	W	<0.060	0.660	0.145	—	—	<0.005*(5)		ND		<0.006	0.015	—
		L	3.263	17.981	8.044	<0.313	<0.658	—(5)				<0.373	1.578	—
Alabama (Chocktaw Co.) (Mobile Co.)	10	W	0.033	0.088	0.071	—	—	<0.005*(6)		ND		<0.006	0.091	—
		L	2.782	13.048	6.168	<0.270	<0.400	—(6)				<0.324	8.696	—
Georgia (Oconee Co.) (Toombs Co.)	10	W	0.047	0.170	0.097	—	—	6.005*(8)	<0.005	0.370	0.124(7)	—	—	<0.006*
		L	3.538	12.067	7.426	<0.249	<0.256	—(8)	<0.282	25.800	9.597(7)	—	0.455	—
Louisiana (Terrebonne Co.)	10	W	0.060	0.220	0.121	0.001	0.002	0.002		ND		0.004	0.010	0.008
		L	6.154	28.529	15.853	0.110	0.357	0.225				0.440	1.316	0.967
Florida (Highland Co.)	10	W	0.055	0.160	0.096	—	—	<0.005*(5)	0.007	0.011	0.008(7)	<0.006	0.008	—(1)
		L	3.924	15.766	8.981	<0.224	<0.505	—(5)	0.519	0.760	0.687(7)	<0.379	0.645	—(1)

W = wet weight; L = lipid weight.
* = All birds reported at this value.
ND = Not detected; value = 0 in computing means.
Numerals in parentheses indicate number of birds in which the compound was not detected.
< quantities taken at face value in computing means.
Means are arithmetic.

TABLE 2.—Intersite comparison of means of DDE and PCB (ppm lipid basis) in dove breast muscle, 1970-71¹

SITE		DDE MEANS		SITE		PCB MEANS	
COUNTY	STATE	ARITHMETIC	GEOMETRIC	COUNTY	STATE	ARITHMETIC	GEOMETRIC
Oconee	Ga.			Terrebonne	La.	15.85	18.32 a
Toombs	Ga.	9.38	5.45 a	Washington	R.I.	14.13	13.76 ab
Madison	Miss.	7.72	4.67 ab	Cape Girardeau	Mo.	13.32	11.54 abc
Highland	Fla.	7.76	4.23 ab	Charleston	S.C.	12.00	11.48 abc
Chocktaw	Ala.			Montgomery	Va.	15.52	11.28 abc
Mobile	Ala.	11.49	3.95 ab	Stewart	Tenn.	12.32	10.84 abc
Cape Girardeau	Mo.	4.98	3.48 abcd	Highland	Fla.	8.98	8.33 bcd
Charleston	S.C.	7.74	3.12 abcd	Chocktaw	Ala.		
Washington	R.I.	3.07	2.82 abcd	Mobile	Ala.	6.17	8.33 bcd
Berks	Pa.	6.00	1.70 abcde	Trigg	Ky.	8.33	7.86 bcd
Trigg	Ky.	0.90	1.12 bcde	Madison	Miss.	8.04	7.48 bcd
Stewart	Tenn.	1.78	1.07 bcde	Oconee	Ga.		
Conway	Ark.	1.73	0.96 cde	Toombs	Ga.	7.43	7.09 bcd
Terrebonne	La.	1.06	0.91 de	Conway	Ark.	7.20	6.80 bcd
Wake	N.C.	1.09	0.90 de	Anne Arundel	Md.		
Anne Arundel	Md.			Price Georges	Md.	5.93	6.02 cd
Prince Georges	Md.	0.96	0.61 e	Wake	N.C.	4.65	4.46 d
Montgomery	Va.	1.47	0.45 e	Becks	Pa.	7.03	3.98 d

¹ The geometric means of any two sites not followed by the same letter are significantly different (Duncan multiple range test).

PESTICIDES IN SOIL

*DDT Residues in Soil, Water, and Fauna from New York Apple Orchards*¹

R. J. Kuhr,² A. C. Davis,² and J. B. Bourke³

ABSTRACT

Five commercial apple orchards which had not been sprayed extensively with DDT since approximately 1960 were surveyed in 1972 for residues of DDT and its metabolites. In addition to the parent compound, DDE and DDD were almost always recovered, but no dicofol was detected in any soil sample. Total residues in the top 6-in. soil layer underneath the trees ranged from 21.8 to 259 lb/acre. Between the rows of trees the levels were considerably lower; they ranged from 7.3 to 78.5 lb/6-in. acre. In one heavily contaminated orchard, researchers also analyzed stream water, stream-bottom mud, and animals. Very low amounts of DDT (0.32 ppb) and DDD (0.042 ppb) were found in the water, and residues in stream-bottom mud totaled less than 1 ppm. Worms, slugs, snails, tadpoles, fingerling fish, and frogs all contained DDT, DDE, and DDD.

Introduction

Residues of DDT and its metabolites persist in many corners of our environment, but one of the largest reservoirs for these chemicals is orchard soil (1). A recent survey which included orchards across the United States revealed a DDT soil load ranging from 0.07 to 245.4 ppm (2). Other, less extensive orchard surveys in the United States, Canada, and Great Britain have indicated residue levels somewhere between these extremes (3-11). In two of these studies, (4, 7), residues of DDT and analogs were also reported for soil insects, slugs, snails, and worms. Most of the animals had accumulated 2 to 4 times as much DDT as was present in their surrounding soil.

In New York, about 72,000 acres of land are currently devoted to commercial apple orchards. Although most of the orchards have not been treated regularly with DDT for more than 10 years, almost all of them had relied on DDT for insect control from approximately 1947 to 1960. During this time, an acre in a typical commercial apple orchard would have been sprayed with 250-400 lb of DDT. The following study was designed to examine how much DDT and metabolites still remained in the soil, how the residue was distributed in the soil, and whether any of the residue had moved into waterways or animal life within the orchard.

Materials and Methods

Five commercial orchards were chosen at random from the heart of New York's apple belt along the southern shore of Lake Ontario. Soil samples were removed from an area near the middle of each orchard. Four of the locations contained trees at least 25 years old, but the trees from orchard G were estimated to be only about 15 years old. Orchard D was sampled in two locations: one at the top of a hill near a stream meandering through the orchard, and the other in a low spot where drainage tile had recently been installed.

At each site, four soil cores were removed from under each of six trees in an area about halfway between the trunk and outer branches. An additional 24 cores were taken from the aisles between the rows of trees. No attempt was made to scrape away surface vegetation or decaying plant debris before sampling. This material was included as part of the upper soil layer. Each cylindrical core consisted of three soil plugs 1½ in. in diameter, 2 in. long, and 4.14 cubic in. in volume. After removal of the 2-in. plug, a second sampler was carefully inserted into the same hole to get the 2- to 4-in. plug;

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the same procedure was repeated for the 4- to 6-in. plug. The 24 plugs from each depth were sealed in a plastic bag until they could be weighed and thoroughly mixed. Only large stones were removed to prevent breakage of glassware during the extraction procedure. Since orchard management (cultivation, vegetative cover, etc.) and soil type and texture varied, it was decided to use unscreened wet soil directly from the orchard and to convert all results to lb residue/2-in. acre for comparative purposes.

Two 100-g subsamples from each bag of soil were weighed separately into 1-liter flasks. Enough water (~40 ml) was added to each flask to make a slurry; into the slurry was poured 600 ml of 3:1 petroleum ether:isopropanol. The flask was placed on a wrist-action shaker and thoroughly agitated for 5 min. After separation, the solvent layer was decanted into a 1-liter separatory funnel and the alcohol was removed with three 150-ml washes of water. The remaining petroleum ether extract was dried over anhydrous sodium sulfate and a portion was injected directly into an Aerograph Hy-Fi Model 550-B gas chromatograph equipped with an electron-capture detector and a 3-ft-by-1/8-in-column containing 5% Dow 11 on 60-80 Gas Chrom-Q at a temperature of 195°C. Recovery from DDT-spiked soil samples averaged 95% and method sensitivity allowed for detection levels above 0.13 lb 2-in. acre. Several other extraction procedures were tried, but none removed more residues than did the method described above. There were no chemicals extracted from the soil which interfered with DDT and DDT-analog peaks from the gas chromatograph. Reported values for soil residues have not been corrected for recovery and represent an average of the two subsamples.

On several occasions, two 1-gal. water samples and two 1-qt bottom mud samples were taken from the stream in orchard D at four different locations. Additional samples were removed from a small bay in the stream where spray tanks were filled and from the end of a drainage tile where it emptied into the stream. The mud samples were extracted as described for soil except that the petroleum ether extract was concentrated before gas chromatography (95% recovery, 0.005 ppm sensitivity limit). Each gallon of water was distributed equally between three 2-liter separatory funnels. To the first funnel was added 150 ml of petroleum ether. After thorough mixing and separation of layers, the petroleum ether was drawn off and transferred to the second funnel. Following extraction of this water, the process was repeated on the third funnel. The entire procedure was reenacted two more times, changing only the sequence of funnels. The combined petroleum ether extracts were dried with anhydrous sodium sulfate and concentrated before gas chromatographic analysis. Spiked recoveries averaged over 98% and method sensitivity was 0.13 ppb.

Orchard D was also the site of animal collections. Worms were obtained by digging soil and turning over surface debris under and between rows of trees in the area where soil samples were removed. Most of the slugs were captured under trees near the stream. Frogs, tadpoles, fingerling fish, and snails were scooped from the stream with a long-handled net. All specimens were brought back to the laboratory where they were washed, weighed, and immediately frozen. This procedure was repeated on two separate occasions, producing a total sample weight of 36.6 g worms, 6.1 g slugs, 107 g frogs, 23.4 g tadpoles, 7.7 g fish, and 11.0 g snails. Each collection was analyzed separately, and results were averaged for the two corresponding samples. Residues were determined after the animals had been freeze-dried and extracted according to the method of Harenc et al. (12). The final hexane extract was analyzed by gas chromatography as described for soil samples. Recovery from spiked tissues averaged 78% and reported values have been corrected to 100%.

Results and Discussion

All soil samples removed from the orchards contained measurable amounts of DDT and DDE; many samples also contained DDD. Although the analysis procedure allowed for detection of dicofol, which has been discovered in other orchard soil (8-10), this DDT metabolite was not picked up in any of the test orchards. The total load of DDT, DDE, and DDD in the top 6 in. of soil and vegetation varied considerably from a high of 258.8 lb/acre under the trees in orchard D to a low of 7.3 lb/acre between the rows of trees in orchard G (Table 1). It is difficult to explain this broad range because the complete spray history of each orchard is unknown. Except for orchard G, the orchards were sampled in areas which had probably been exposed to regular DDT treatments for more than 10 years. Undoubtedly, some of the variation was due to differences in soil type, soil structure, chemical makeup, physical conditions, and cultivation practices. However, only a very small portion of the residues are probably due to the DDT spray used occasionally after 1960 because the total application during this period probably did not amount to more than 2-3 lb/acre.

Based on previous studies, the horizontal and vertical distribution of residues within the soil was not surprising. For example, more residues were located under the trees than between the rows of trees (Table 1). This has been an almost universal finding in orchard surveys (5, 6, 9, 13, 14). With the exception of the tiled region between the trees of orchard D, there was a general trend of decreasing contamination with increasing soil depth. A change in residue profile for the tiled region was probably due to the turnover and mixing of soil that occurred when the drainage system was installed. In highly contaminated orchards (D-Hill, C, and P),

75-85% of the total residue still remained in the top 2-in. layer. In the other orchards these values were inclusive for the upper 4 in. of soil. Previous orchard surveys which probed deeper than 6 in. have yielded similar results (6, 10, 14), although in these instances samples were taken during or shortly after DDT treatment. The results in Table 1, obtained from orchards that had not been sprayed regularly with DDT for approximately 13 years, indicate that there has been very little downward movement of DDT during this time.

If the total residue distribution is broken down into individual compounds, an interesting consistent observation can be made. At almost every site, the percentage of the total 6-in. residue found in the top 2 in. of soil was nearly the same for DDT and DDE. On the other hand, where DDD was present, its distribution within the top 2 of 6 in. was almost always relatively higher. This higher soil load of DDD in the upper layer is probably due, in part, to several factors. First, during the time of DDT usage, most orchard owners applied some DDD (Rhothane) to control red-banded leaf rollers; infestations of this insect varied greatly from

season to season and from orchard to orchard, and the use of DDD varied accordingly. The initial deposition of DDD may have migrated more slowly down into the 4- and 6-in. soil layers than did the DDT. Secondly, microorganisms in the decaying vegetation present in the top soil layer may have converted more DDT to DDD. Orchard sites which contained no detectable levels of DDD could have experienced a minimum amount of DDT-to-DDD conversion in the soil, coupled with a minimum application of DDD.

Of the total residue located in all the orchards at every soil depth, 70-90% persisted as DDT. With some exceptions, the remainder was divided between DDE and DDD so that DDE levels were always approximately equal to or greater than DDD levels. This distribution fits a pattern that seems common to many orchards (5, 8-10, 15).

Turning to the animal life collected from orchard D, it is apparent that residues of DDT, DDE, and DDD were present in every species captured (Table 2). Worms and slugs which were in direct contact with the heavily

TABLE 1.—Residues of DDT, DDD, and DDE in soil from five New York apple orchards

SOIL LEVEL, IN.	RESIDUES EXPRESSED AS LB/2-IN. ACRE ¹							
	UNDER TREES				BETWEEN ROWS OF TREES			
	DDT	DDD	DDE	TOTAL	DDT	DDD	DDE	TOTAL
ORCHARD D, TILED REGION								
0-2	152.0	8.9	14.4	175.3	16.4	4.2	2.1	22.7
2-4	38.3	0.3	4.5	43.1	16.2	0.9	3.4	20.5
4-6	35.7	0.7	4.0	40.4	24.0	4.9	6.4	35.3
0-6	226.0	9.9	22.9	258.8	56.6	10.0	11.9	78.5
ORCHARD D, HILL REGION								
0-2	162.0	14.4	12.3	188.7	40.9	3.0	6.7	50.6
2-4	27.5	1.1	3.4	32.0	6.5	0.3	1.4	8.2
4-6	13.7	1.5	1.9	17.1	3.0	0.1	0.4	3.5
0-6	203.2	17.0	17.6	237.8	50.4	3.4	8.5	62.3
ORCHARD C								
0-2	135.0	19.5	9.7	164.2	42.7	8.5	10.2	61.4
2-4	15.8	0.5	1.4	17.7	9.1	1.2	2.0	12.3
4-6	9.1	0.0	1.1	10.2	2.5	0.0	0.7	3.2
0-6	159.9	20.0	12.2	192.1	54.3	9.7	12.9	76.9
ORCHARD P								
0-2	40.6	0.0	9.4	50.0	22.7	0.0	8.8	31.5
2-4	17.4	0.0	5.0	22.4	5.5	0.0	2.3	7.8
4-6	15.2	0.0	2.6	17.8	1.8	0.0	0.6	2.4
0-6	73.2	0.0	17.0	90.2	30.0	0.0	11.7	41.7
ORCHARD Y								
0-2	17.4	3.0	4.4	24.8	8.2	0.0	3.8	12.0
2-4	10.0	1.2	3.1	14.3	10.6	0.0	4.1	14.7
4-6	5.8	0.0	1.6	7.4	4.0	0.0	1.8	5.8
0-6	33.2	4.2	9.1	46.5	22.8	0.0	9.7	32.5
ORCHARD G								
0-2	8.4	0.6	1.6	10.6	3.2	0.0	0.7	3.9
2-4	6.1	0.0	1.1	7.2	1.4	0.0	0.6	2.0
4-6	3.6	0.0	0.4	4.0	0.9	0.0	0.5	1.4
0-6	18.1	0.6	3.1	21.8	5.5	0.0	1.8	7.3

¹ To convert values for individual 2-in. soil layers to approximate ppm wet weight, multiply by 1.5.

TABLE 2.—*Residues of DDT, DDD, and DDE in animals collected from Orchard D*

ANIMAL	RESIDUES IN PPM BASED ON WET-WEIGHT				RESIDUES IN PPM BASED ON DRY-WEIGHT			
	DDT	DDD	DDE	TOTAL	DDT	DDD	DDE	TOTAL
Earthworm	21.7	4.0	5.1	30.8	106	17.6	24.0	147.6
Slug	5.6	5.2	2.6	13.4	28.1	23.9	12.1	64.1
Frog	2.8	1.8	2.2	6.8	13.3	10.0	10.9	34.2
Tadpole	1.7	1.0	0.6	3.3	16.5	9.0	5.1	30.6
Fish	0.5	0.5	0.6	1.6	3.1	3.0	3.8	9.9
Snail	0.8	0.5	0.3	1.6	1.8	1.5	0.7	4.0

contaminated soil in the hill region of orchard D contained the largest quantities of residues on a wet-weight basis. However, the amounts did not approach those found in the upper 6 in. of soil. Other studies have shown that worm and slug residues were considerably higher than surrounding soil residues (4, 7), although in such instances the soil load was much less than that of orchard D.

Fittingly, an intermediate contamination level was found in frogs. These animals were exposed to residues on land and in the orchard's small stream. Finally, fingerling fish, tadpoles, and snails contained relatively small quantities of DDT, DDE, and DDD. Unlike the land inhabitants, however, the stream dwellers obviously were capable of concentrating residues from their surroundings. Although none of the water collected contained measurable amounts of DDE, an average of 0.32 ppb of DDT and 0.042 ppb of DDD was found in stream samples. Contamination was slightly higher in water collected from the stream bay and the drainage tile. The increase in the tile water may have resulted from its intimate contact with soil residues as it percolated slowly through the soil column. Interestingly, there was a general increase in the quantity of residues present in water as the season progressed from early spring to early fall. There are several possible reasons for this: e.g., changes in flow rate, volume of flow, contribution from ground water. Contamination of the mud below the stream was considerably higher than that in the water; average residues were 0.08 ppm DDT, 0.06 ppm DDE, and 0.12 ppm DDD. This very low presence of DDT and analogs in water and their concentration in bottom mud and aquatic animal life has been well documented by other studies (1).

One noticeable difference between soil residues and animal residues was in the respective amounts of DDT and its degradation products. Only 30-50% of the total contamination in animals was due to DDT itself; in soil, the parent compound represented 70-90% of the total residue. The larger proportion of DDE and DDD in animals was probably due to an increase in DDT metabolism by the animals.

Other investigations have shown that plants grown on DDT-rich soil tend to accumulate DDT residues, although not to the extent of animal life (1). A small sample of dandelions was removed from under the trees of orchard D and thoroughly washed with distilled water. Following aqueous homogenization of a 100-g sample, the plants were extracted and analyzed using the same method described for soil samples. Residues on a wet-weight basis were 4.2 ppm DDT, 2.6 ppm DDD, and 0.86 ppm DDE.

Conclusions

Despite the fact that commercial apple orchards in upstate New York have not received regular DDT treatments for about 13 years, their soil is still contaminated with DDT and some of its metabolites. Qualitative estimates and calculated guesswork indicate that, in one particular orchard, approximately half the DDT applied from 1947 to 1960 continues to persist in the soil as DDT, DDD, and DDE. However, results suggest that other orchard soils contain considerably less material even though they were probably all originally exposed to similar spray schedules.

Within one heavily resided orchard, DDT and analogs were detected in all animal species captured and in a small sample of growing vegetation. Many of these animals had been removed from a stream which trickled through the heart of the orchard. Samples of stream water and bottom mud contained minute quantities of DDT residues, implying that movement of the insecticide from the soil into the waterway was not excessive. In fact, the low levels present in the stream environment coupled with the high soil load suggests that the DDT residues are present in a relatively static situation, moved about primarily by animal life and redistributed principally as part of a mobile food chain.

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*Chlorinated Insecticide Residues in Kentucky Burley Tobacco: Crop Years 1963-72*¹

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ABSTRACT

Kentucky Burley tobacco representing crop years 1963-70 was sampled from the storage facilities of the Burley Tobacco Growers Cooperative Association and from auction warehouses in Kentucky in 1968-72. Analyses of these samples in 1972 showed 100% contained DDT-TDE; 96%, dieldrin; 31%, endrin; and 4%, toxaphene. Endosulfan residues were first detected in a few samples of the 1968 tobacco but were present in 100% of the 1970 and 1972 tobacco, and in 83% of the 1971 crop. Average total chlorinated insecticide residues in tobacco produced during 1963-65 were approximately 20 ppm, and for that produced during 1966-69, levels were about 50 ppm. Levels dropped markedly in 1970 to 12 ppm, declined to 9 ppm in 1971, and then to 4 ppm in 1972. During the years when residues were highest, DDT-TDE constituted over 90% of the total, but declined so rapidly after 1968 that they accounted for only 8% of the total chlorinated insecticide residues in the 1972 tobacco; 90% of the residues on the 1972 tobacco was endosulfan. The relationships of area and year of production to the levels of chlorinated insecticide residues on Burley tobacco are discussed.

Introduction

As of this printing, no official pesticide tolerances on tobacco have been established by the U.S. Government. Whether this situation will change is uncertain. However, it is likely that some action will be taken to control the levels of pesticides on future tobacco crops, either by establishing tolerances or by limiting the kinds and/or amounts of chemicals which may be used. The latter control already applies to DDT and TDE; their registra-

tion for use on tobacco was cancelled prior to the 1970 growing season.

The future of pesticide tolerances on tobacco may be elucidated to some degree by examining recent action of the West German Government (1). Legislation is pending which would place tolerances on approximately 80 different pesticides used on tobacco. Certain chemicals, including the chlorinated insecticides dieldrin, endrin, chlordane, and heptachlor, could not be present at any level, and thus could not be used in any manner for the control of tobacco pests. Other residues would be allowed at low levels, as exist in general environmental contamination, for example. But the acceptable quantities are so low that their use as a pesticide on tobacco probably would be prohibited. Examples of common chlorinated insecticides and their proposed tolerances are DDT-TDE and analogs, 0.1 ppm; toxaphene, 0.1 ppm; and endosulfan, 0.5 ppm.

For most other insecticides commonly used on tobacco the tolerances are usually below 1.0 ppm. However, the tolerance for carbaryl, a carbamate, is 2.5 ppm and for malathion, an organophosphorus compound, it is 3.0 ppm. Although the legislation has not been passed by the West German Government it is quite possible that the final tolerances will remain just this stringent.

Much of the tobacco produced in the United States contains levels of pesticide residues which would not conform to the pending regulations mentioned above. Several studies (2, 3, 4) have shown that tobacco of commercial cigarettes contains residues of chlorinated insecticides much greater than the tolerances proposed in West Germany.

A comprehensive study of the chlorinated insecticide content of Kentucky Burley tobacco has not been made;

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its contribution to the residue levels in United States tobacco products is not known. The current study was conducted to determine the levels of chlorinated insecticides in Kentucky Burley tobacco produced in previous years but still available for sale, and to evaluate the effect of changing insecticide use on the levels and kinds of insecticides in tobacco. These factors were studied in relation to grades of tobacco and to areas in which the tobacco was grown.

Methods and Materials

Burley tobacco samples were collected from tobacco held in storage by the Burley Tobacco Growers Co-operative Association (Burley Tobacco Pool) and from auction warehouses in Kentucky. Tobacco in the Burley Tobacco Pool represents many crop years since it is purchased annually by the Federal Government as part of the U.S. Department of Agriculture price control program. This tobacco, still for sale, is stored at various locations in Kentucky and the storage site generally contains crops produced only in that area. It was possible, then, to compare residue levels with the location of crop production for 1963 through 1970, those crop years for which tobacco was available from the Burley Tobacco Pool. This was evaluated in greater detail in studies in which Burley tobacco was taken from the auction markets at the end of the growing seasons in 1968 through 1972.

SAMPLING

Burley Tobacco Pool

Tobacco stored in the Burley Tobacco Pool is in the hand form contained in a hogshead filled with approximately 1000 pounds of tobacco. Because tobacco in one hogshead could be from various farms subjected to diverse insecticides, a sampling method was developed that would yield a small portion of tobacco containing residues representative of the entire hogshead of tobacco.

A 1-in.-by-48-in. core of tobacco was taken from each hogshead at a point 12 inches from the outer edge of the upright container. Analysis of a thoroughly mixed sample collected in this manner gave results comparable to those obtained by analyzing 100 10-g subsamples from a hogshead of tobacco which has been chopped and mixed by commercial means (5).

Four different storage sites in Kentucky were selected for sampling, one each in areas II, III, IV, and V (Fig. 1). The specific warehouses were selected because they contained tobacco produced in that area, and because of the large number of crop years for which tobacco was available. When possible, three different grades of tobacco from each crop year were collected from each sampling site. The grades preferred were B3F, C4F, and X3F because they contain leaves approximating the top, middle, and bottom portions of

the tobacco plant. When these grades were not available, similar grades were sampled; in some cases, similar grades were not available so no sample was taken.

For crop years 1965 and 1967-70, 2 hogsheads of each grade were sampled from areas II-V, making a total of 8 hogsheads per grade per year. Grade B, 1963 tobacco was not available in areas III, IV, and V; nor were B and C grades of the 1964 crop in area III. Sampling of the 1966 crop was complete except that there was no grade B tobacco in area II. Two core samples were taken from each hogshead; the tobacco from each was cut into cigarette-size particles and thoroughly mixed. Two 10-g subsamples from each core were analyzed.

Auction Warehouse Tobacco

For sampling purposes, Kentucky was divided into six areas (Fig. 1) and tobacco was collected from as many counties and individual farms within the area as were available at the time of collection. Three hands of tobacco from each farm were collected: one hand each from the top, middle, and bottom leaves of the tobacco plant. A composite sample also was taken which consisted of equal quantities of the top, middle, and bottom leaves. The four samples were individually cut into cigarette-size particles, combined with like samples from the same area, and thoroughly mixed. Two 10-g subsamples were removed for analysis. The numbers of counties and farms represented for each area and crop year are shown in Figure 1. Separate analyses were performed on the top, middle, bottom, and composite leaves from each area for the crop years 1968-72.

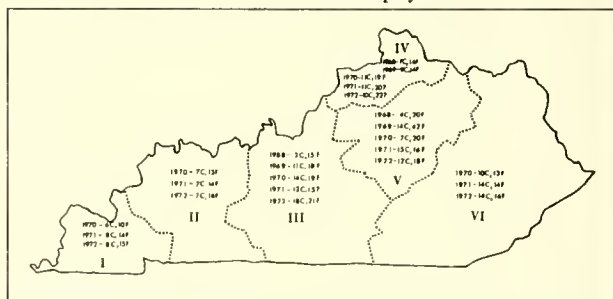


FIGURE 1.—Map of Kentucky showing areas from which tobacco samples were taken, and number of counties (C), and farms (F) represented by auction warehouse samples for indicated crop years.

All analyses were performed during 1972 and early 1973 using identical procedures for all samples. Tobacco from the Burley Tobacco Pool was stored in hogsheads, as stated earlier; tobacco samples collected annually from the auction markets were placed in plastic bags and held in the laboratory until analyzed.

Extraction and Cleanup

Tobacco samples were extracted overnight in a soxhlet apparatus with a 9:1 mixture of chloroform and methanol (6). Florisil column cleanup of the extracts

for analysis of all chlorinated insecticides except endosulfan was identical to that described by Skrentny and Dorough (7). A deactivated florasil column, 2% water, was used for extracts being analyzed for endosulfan (4).

Gas Chromatography

Two Varian Aerograph Model 1700 instruments equipped with tritium electron capture detectors were used in these experiments. One chromatograph contained a 6 ft.-by-1/8-in. pyrex column packed with a 1:1 mixture of 4% SE30 and 6% QFI on 80/90-mesh Anakrom ABS. The temperatures of the injector, column, and detector were 215°C, 200°C, and 210°C. A column of the same size containing 5% OV-210 on 100/200-mesh Chromosorb W-HP was used with the other instrument. The injector temperature was 210°C, the column was 180°C, and the detector was 210°C. Nitrogen, 40 ml/min, served as the carrier gas for both instruments.

Chromatograms of the various insecticide standards and tobacco extracts obtained with these gas chromatographic systems are illustrated in the paper by Dorough and Gibson (4). Minimum detectable levels of the insecticides in tobacco were 0.01 ppm for *p,p'*-DDT, *o,p*-DDT, *p,p'*-TDE, *o,p*-TDE, *p,p'*-DDE, dieldrin, endrin, chlordane, endosulfan I and II; 0.05 for endosulfan sulfate; and 0.5 ppm for toxaphene. Average recoveries of the insecticides when added to control tobacco exceeded 90% for all materials except endosulfan sulfate and toxaphene; recoveries of these insecticides averaged 88 and 80%, respectively. The extraction procedure reported here removed from 25 to 80% more chlorinated insecticide residues from tobacco than did certain

other extraction methods commonly employed (7). Data were not corrected for recovery. Mass spectrometry (Finnigan Model 1015C GC/MS System) was used to confirm the findings.

Results and Discussion

FREQUENCY OF OCCURRENCE

DDT and/or TDE residues were present at detectable levels in all tobacco samples analyzed in this study (Table 1). During the period from 1963 to 1969, all samples except some of the 1964 tobacco crop contained residues in excess of 5 ppm and many contained over 25 ppm of DDT-TDE. Over 80% of the tobacco produced in 1966-69 contained DDT-TDE residues in excess of 25 ppm, but in the 1971 crop the residues were less than 5 ppm in 50% of the samples. DDT-TDE residues on the 1972 crop were below 1 ppm.

Dieldrin also was present on most of the tobacco sampled from the Burley Tobacco Pool and from the auction warehouses. Among samples from the 1964 and 1970 tobacco crops, 90% and 96% contained dieldrin. All other samples from the Burley Tobacco Pool were contaminated with this insecticide. All tobacco collected from the auction warehouses contained dieldrin up to the 1972 crop, in which 33% of the samples were free of detectable levels of residues.

Endrin was the third most common chlorinated insecticide on tobacco: only the 1968 and 1969 auction market samples did not show endrin residues at some level. Unlike dieldrin, endrin was not detected in 100% of the samples for any crop produced between 1963 and 1972. Usually, less than half the samples analyzed for any given crop year contained this material. Also, the

TABLE 1.—Frequency of occurrence of chlorinated insecticide residues in Kentucky Burley tobacco.

CROP YEAR	PERCENT OF SAMPLES CONTAINING							
	TOXAPHENE	ENDOSULFAN	DIELDRIN	ENDRIN	TOTAL DDT-TDE			
					0-1 PPM	1-5 PPM	5-25 PPM	25+ PPM
BURLEY TOBACCO POOL SAMPLES								
1963	17	0	100	72	0	0	100	0
1964	0	0	90	60	0	25	40	35
1965	4	0	100	29	0	0	71	29
1966	4	0	100	45	0	0	9	91
1967	0	0	100	21	0	0	17	83
1968	0	0	100	25	0	0	13	87
1969	8	96	100	30	0	0	4	96
1970	8	100	96	17	33	50	17	0
AUCTION WAREHOUSE SAMPLES								
1968	0	35	100	0	0	0	0	100
1969	0	35	100	0	0	0	0	100
1970	17	100	100	17	0	60	33	7
1971 ¹	0	83	100	50	50	17	33	0
1972	0	100	67	33	100	0	0	0

¹ 83% of these samples also contained chlordane.

occurrence of endrin on tobacco was not evenly distributed across the state as were DDT and dieldrin residues. For example, of the 64 hogsheads containing endrin-contaminated tobacco (a total of 180 were sampled for 1963-70), 39 were in area II; 13, in area III; 5, in area IV; and 7, in area V (Fig. 1). Areas I and VI were not included in the Burley Tobacco Pool experiments because of the relatively low quantities of tobacco produced in these areas.

Toxaphene was not a general contaminant of tobacco produced in Kentucky during 1963-72, but did occur sporadically and sometimes at very high levels. Residues of toxaphene were not detected on any samples of tobacco collected from areas IV, V, and VI. Low levels of chlordane were present on 83% of the auction warehouse samples collected in 1971 but none was detected in any other tobacco samples.

Endosulfan residues appeared on about one third of the tobacco sampled from the auction markets in 1968 and 1969. In subsequent years endosulfan was a general contaminant, present in all 1970 and 1972 samples and in 83% of the 1971 auction warehouse tobacco. Its high rate of occurrence on Burley tobacco produced after 1968 was verified by analysis of the Burley Tobacco Pool samples.

Table 1 shows some differences in the frequency with which insecticides occurred on the Burley Tobacco Pool compared to samples from the auction warehouses for the same crop year. These differences become even more apparent in later discussions which consider specific levels. Generally, the Burley Tobacco Pool samples would more accurately represent average residue levels for a given area, or for the State as a whole. This is

because a single hogshead of tobacco may contain tobacco produced by as many as 20 farmers at any location within any designated area.

For most crop years from 1963 to 1970, 8 hogsheads were sampled from each area. This would represent tobacco from 100 to 200 producers from that area each year and the resulting data would be indicative of the residues for the area as a whole.

On the other hand, samples from the auction markets were taken from three or four specific warehouses within an area, usually representing crops produced by only 10 to 20 farmers for each area and crop year. Moreover, areas I and VI (Fig. 1) were included in the auction warehouse studies and information from these areas are included in all data reflecting average residue levels on tobacco on a statewide basis (Tables 1, 3, 7, 9). Similar data from the Burley Tobacco Pool samples (Tables 1, 2, 6, 8) would not include tobacco from areas I and VI. These factors could certainly be responsible, at least in part, for the differences observed in insecticide occurrence and levels on tobacco samples taken from the Burley Tobacco Pool and the auction warehouses for the same crop years, although the differences were not dramatic.

TOTAL CHLORINATED INSECTICIDE RESIDUES ON STATEWIDE BASIS

The average levels of chlorinated insecticide residues on Burley tobacco produced in Kentucky during 1963-72 were of three definite magnitudes. Tobacco crops produced in 1963-65 contained average residues of 20 ppm; tobacco produced in 1966-69, about 50 ppm; and that produced after 1969, less than 15 ppm (Tables 2, 3). Total chlorinated insecticide residues continued to

TABLE 2.—Chlorinated insecticide residues, ppm, in tobacco sampled from Kentucky Burley Tobacco Pool¹

CROP YEAR	TOTAL DDT-TDE'S	DIELDRIN	ENDRIN	ENDOSULFAN	TOXAPHENE	TOTAL RESIDUES
1963	12.26	0.40	0.36	0	7.53	20.55
1964	20.06	0.32	0.30	0	0	20.68
1965	25.55	0.46	0.22	0	0.28	26.51
1966	52.99	0.84	0.25	0	0.67	54.75
1967	50.62	0.70	0.19	0	0	51.51
1968	50.96	0.84	0.14	0	0	51.95
1969	53.02	0.86	0.28	0.86	0.68	55.70
1970	3.54	0.26	0.06	2.68	1.92	8.46

¹ Values are averages of analyses of all hogsheads sampled for indicated year. Number of hogsheads sampled: 18 in 1963, 20 in 1964, 22 in 1966, and 24 in 1965 and 1967-70.

TABLE 3.—Chlorinated insecticide residues, ppm, in Burley tobacco sampled from auction warehouses in Kentucky¹

CROP YEAR	TOTAL DDT-TDE'S	CHLORDANE	DIELDRIN	ENDRIN	ENDOSULFAN	TOXAPHENE	TOTAL RESIDUES
1968	54.68	0	0.61	0	0.23	0	55.52
1969	53.24	0	0.94	0	0.30	1.62	56.10
1970	8.16	0	0.59	0.07	4.19	1.14	14.15
1971	3.18	0.27	0.12	0.12	4.60	0.46	8.75
1972	0.29	0	0.04	0.06	4.10	0	4.49

¹ Values are averages of all analysis of samples collected for indicated year.

decline steadily after the very significant drop which occurred in the 1970 tobacco crop, averaging less than 5 ppm in 1972 (Table 3). DDT-TDE constituted over 90% of the total chlorinated insecticide residues on tobacco when residues were at their peak level during 1966-69. The decline in later years was brought about largely by a reduction in levels of these residues. The most significant point was the low level to which they declined: from 53 ppm in 1969, to 0.3 ppm in 1972. Dieldrin residues on Burley tobacco ranged from 0.26 to 0.94 ppm on a statewide basis in the 1963-70 tobacco crops (Tables 2, 3). The average level during this period for tobacco sampled from the Burley Tobacco Pool was 0.60 ppm (Table 2). Endrin levels on the 1963-65 tobacco, 0.2-0.4 ppm, were approximately equal to those of dieldrin. However, endrin residues remained about the same through 1969, whereas levels of dieldrin increased during the late sixties.

Analysis of the 1968 and 1969 auction market samples did not reveal any endrin residues, but low levels were present on the 1970-72 tobacco (Table 3). Like the

DDT-TDE's, levels of dieldrin and endrin steadily decline on tobacco produced after 1969; the average level of dieldrin and endrin on the 1972 crop was 0.04 ppm and 0.06 ppm, respectively.

As stated earlier, toxaphene was not present on a large number of samples. However, some individual samples contained more than 100 ppm of this insecticide, causing its levels in tobacco, considered statewide, to appear significant in some cases (Table 2). The toxaphene residues shown in Table 2 were in tobacco from only 9 of the 180 hogsheads sampled: 4 from area II, and 5 from area III. Therefore, toxaphene was not a general contaminant of Kentucky Burley tobacco, nor of tobacco in areas II and III. No toxaphene was detected in 79 of the 88 hogsheads from these areas.

Endosulfan first appeared on the 1968 tobacco crop, but on a statewide basis averaged only 0.23 ppm (Table 3). In 1969, the levels remained fairly low, less than 1 ppm (Tables 2, 3), but increased markedly to over 4 ppm in the 1970-72 Burley tobacco (Table 3). Its

TABLE 4.—Chlorinated insecticide residues, ppm, in tobacco sampled from Kentucky Burley Tobacco Pool according to area of production and crop year

AREA, YEAR	<i>p,p'</i> - DDT	<i>o,p'</i> - DDT	<i>p,p'</i> - TDE	<i>o,p'</i> - TDE	<i>p,p'</i> - DDE	TOTAL DDT-TDE	ENDO- SULFAN	DIELDRIN	ENDRIN	TOXA- PHENE	TOTAL RESIDUES
Area II											
1963	3.16	0.93	6.17	1.45	0.40	12.11	0	0.68	0.83	29.32	42.93
1964	5.57	0.71	15.78	4.13	0.66	26.85	0	0.55	0.96	0	28.36
1965	9.94	1.14	28.96	8.71	0.80	49.54	0	1.03	0.84	0	51.42
1966	27.55	2.72	34.60	10.13	1.63	76.64	0	1.26	0.96	0	78.86
1967	32.11	3.15	39.29	7.31	2.15	84.02	0	1.39	0.75	0	86.16
1968	19.22	1.91	41.14	7.02	1.70	70.99	0	1.47	0.50	0	72.96
1969	24.06	2.28	45.84	8.93	1.60	80.71	0.94	1.22	1.05	0	83.92
1970	1.48	0.18	3.12	1.81	0.14	6.74	3.40	0.31	0.25	7.68	18.37
Area III											
1963	1.39	0.19	9.15	1.74	0.14	12.61	0	0.22	0.61	0.81	14.24
1964	5.62	0.90	13.44	2.90	0.64	23.51	0	0.36	0.20	0	24.07
1965	5.45	0.77	12.21	2.61	0.54	21.58	0	0.34	0.02	1.10	23.05
1966	31.68	2.78	24.16	4.34	1.98	64.94	0	0.68	0.02	2.68	68.32
1967	15.36	1.68	26.04	6.01	1.33	50.42	0	0.62	0	0	51.04
1968	16.97	1.64	22.07	4.21	0.88	45.77	0	0.65	0.10	0	46.52
1969	17.64	2.04	32.47	8.50	1.34	62.00	0.88	1.09	0.05	2.70	66.71
1970	1.68	0.22	1.41	0.35	0.07	3.74	3.04	0.26	0.01	0	7.04
Area IV											
1963	0.93	0.09	7.83	2.00	0.13	10.97	0	0.31	0.01	0	11.28
1964	7.08	0.79	5.48	1.06	0.78	15.16	0	0.10	0	0	15.26
1965	6.02	0.72	4.79	1.23	0.74	13.50	0	0.19	0	0	13.68
1966	16.67	1.64	16.95	3.90	1.57	40.72	0	0.44	0.01	0	41.17
1967	12.53	1.08	14.68	2.31	1.04	31.64	0	0.21	0	0	31.85
1968	14.15	1.38	12.97	2.72	1.29	32.50	0	0.47	0	0	32.98
1969	14.16	1.45	12.78	2.37	0.99	31.74	0.88	0.52	0	0	33.14
1970	0.17	0.02	1.36	0.34	0.01	1.90	1.40	0.27	0	0	3.56
Area V											
1963	3.16	0.53	7.14	2.11	0.40	13.33	0	0.39	0.02	0	13.74
1964	3.62	0.42	8.13	2.09	0.45	14.72	0	0.27	0.04	0	15.03
1965	3.77	0.47	10.24	2.67	0.44	17.58	0	0.30	0.01	0	17.90
1966	12.28	1.42	12.18	2.93	0.86	29.66	0	1.00	0	0	30.66
1967	9.40	1.11	20.15	4.65	1.11	36.41	0	0.57	0	0	36.98
1968	23.26	2.07	24.56	3.60	1.06	54.56	0	0.78	0	0	55.34
1969	14.38	1.28	18.11	3.16	0.69	37.61	0.76	0.60	0	0	38.98
1970	0.54	0.09	0.77	0.34	0.05	1.80	2.86	0.21	0	0	4.86

ubiquitous nature and the fact that many samples contained 10 to 20 ppm endosulfan is not unlike the situation in the early 1960's regarding DDT-TDE residues on tobacco.

RESIDUES IN RELATION TO AREA OF PRODUCTION AND CROP YEAR

Of the four areas in Kentucky from which Burley Tobacco Pool samples were collected (Fig. 1), the highest levels of total chlorinated insecticide residues were always found in tobacco from area II (Table 4). The average level for the 8-year period was 58 ppm. During 6 of the 8 years, tobacco from area IV contained the lowest quantity of insecticide residues; area V tobacco

was lowest the other 2 years. Eight-year averages for areas IV and V were 23 ppm and 27 ppm, respectively. Tobacco from area III always contained residues higher than those from area IV and, except for the 1968 crop, lower than area V. Total chlorinated insecticide residues on area III tobacco averaged 38 ppm for the crop years 1963-70.

Analysis of the auction warehouse tobacco samples showed that area I tobacco was similar in insecticide content to that from area II (Table 5). Area VI tobacco proved to be lower in chlorinated insecticide content than tobacco from any of the areas in which samples were collected in 1970-72. Average total residues were

TABLE 5.—Chlorinated insecticide residues, ppm, in Burley tobacco sampled from auction warehouses in Kentucky according to area of production and crop year

AREA, YEAR	<i>p,p'</i> -DDT	<i>o,p'</i> -DDT	<i>p,p'</i> -TDE	<i>o,p'</i> -TDE	<i>p,p'</i> -DDE	TOTAL DDT-TDE	CHLOR-DANE	ENDO-SULFAN	DIEL-DRIN	EN-DRIN	TOXA-PHENE	TOTAL RESIDUES
Area I												
1970	4.29	0.48	8.30	1.54	0.21	14.82	0.00	14.63	2.22	0.00	6.62	38.29
1971	2.73	0.79	5.21	1.21	0.21	10.15	0.15	13.78	0.15	0.55	0	24.78
1972	0.29	0.11	0.10	0	0.03	0.53	0	7.45	0.08	0.07	0	8.13
Area II												
1970	5.12	0.38	23.49	5.37	0.20	34.56	0	3.21	0.71	0.63	0	39.11
1971	1.52	0.40	5.79	1.23	0.09	9.03	0.08	10.85	0.14	0.62	0	20.72
1972	0.20	0.07	0.09	0	0.02	0.38	0	5.46	0.02	0.43	0	6.29
Area III												
1968	1.17	0.56	24.93	4.63	0.07	31.76	0	0	0.73	0	0	32.49
1969	16.14	2.06	39.02	9.97	0.88	68.07	0	0	1.23	0	4.07	73.37
1970	0.24	0.16	1.70	0.39	0	2.49	0	2.28	0.10	0	0	4.87
1971	0.12	0.11	1.27	0.28	0	1.78	0.06	2.81	0.19	0.25	0	5.09
1972	0.19	0.07	0.06	0	0.02	0.34	0	3.65	0	0	0	3.99
Area IV												
1968	35.23	5.32	12.15	0.92	0.71	54.33	0.00	0.00	0.27	0.00	0.00	54.60
1969	14.41	2.16	19.33	4.77	0.97	41.64	0	0	0.99	0	0	42.63
1970	0.22	0.11	0.80	0.29	0	1.42	0	2.93	0.64	0	0	4.99
1971	0.11	0.09	0.13	0.03	0.01	0.37	0.04	0.89	0.07	0	0	1.37
1972	0.08	0.03	0.04	0.01	0.01	0.16	0	2.59	0.07	0	0	2.85
Area V												
1968	16.69	2.89	20.57	3.87	0.95	47.94	0	0.90	0.90	0	0	49.74
1969	19.83	2.19	17.29	2.76	0.83	42.90	0	1.06	0.86	0	0	44.82
1970	2.95	0.69	8.86	1.63	0.09	14.22	0	3.26	0.47	0	0	17.95
1971	0.07	0.05	0.11	0.03	0.01	0.27	0	1.39	0.08	0	0	1.78
1972	0.09	0	0.04	0	0	0.13	0	1.91	0.04	0	0	2.08
Area VI												
1970	0.74	0.17	1.27	0.36	0	2.54	0	0.55	0.05	0	0	3.14
1971	0.05	0.09	0.05	0.01	0	0.16	0.05	0	0.02	0	0	0.25
1972	0.06	0	0.04	0	0	0.10	0	0.12	0	0	0	0.22

TABLE 6.—Endosulfan components in tobacco sampled from Kentucky Burley Tobacco Pool

GRADE	PPM AND PERCENTAGE OF TOTAL ON TOBACCO FOR CROP YEARS ¹							
	1969				1970			
	ENDOSULFAN				ENDOSULFAN			
	I	II	SULFATE	TOTAL	I	II	SULFATE	TOTAL
B3F	0.10	0.35	0.19	0.64	0.22	1.17	1.12	2.51
C4F	0.08	0.31	0.44	0.83	0.23	1.13	1.30	2.66
X3F	0.14	0.34	0.65	1.13	0.24	1.17	1.32	2.73
Average	0.11 (12.6)	0.33 (37.9)	0.43 (49.5)	0.87	0.23 (8.8)	1.16 (44.1)	1.25 (47.1)	2.63

¹ Values represent average of analysis of tobacco from 8 hogsheads: 2 of each grade each year from each area, II-V, described in Fig. 1.

TABLE 7.—Endosulfan components on Burley tobacco sampled from auction markets in Kentucky

CROP YEAR AND INSECTICIDE	PPM AND PERCENTAGE OF TOTAL ON LEAVES FROM DIFFERENT PORTIONS OF PLANT ¹				
	TOP	MIDDLE	BOTTOM	AVERAGE	COMPOSITE ²
1970					
Endosulfan I	0.65	0.80	0.86	0.77 (18.9)	0.78 (17.4)
Endosulfan II	1.13	1.15	1.22	1.17 (28.5)	1.38 (30.8)
E. Sulfate	1.86	2.33	2.30	2.16 (52.6)	2.32 (51.8)
Total	3.64	4.28	4.38	4.10	4.48
1971					
Endosulfan I	0.51	0.88	0.90	0.76 (18.5)	0.80 (16.1)
Endosulfan II	2.51	1.03	1.56	1.70 (41.4)	2.27 (45.8)
E. Sulfate	1.40	1.41	2.15	1.65 (40.1)	1.89 (38.1)
Total	4.42	3.32	4.61	4.11	4.96
1972					
Endosulfan I	0.29	0.31	0.66	0.42 (8.3)	0.25 (7.1)
Endosulfan II	0.79	1.13	2.64	1.52 (16.1)	0.81 (23.0)
E. Sulfate	2.60	3.57	3.12	3.10 (75.6)	2.47 (69.9)
Total	3.68	5.01	6.42	5.04	3.53

¹ Average of all samples analyzed for indicated year.² Sample consisting of equal quantities of top, middle, and bottom leaves.TABLE 8.—Chlorinated insecticide residues, ppm, in different grades of tobacco sampled from Kentucky Burley Tobacco Pool¹

CROP YEAR	GRADE			AVERAGE
	B	C	X	
1963	14.82	37.45	9.38	20.55
1964	29.78	17.83	14.43	20.68
1965	24.88	24.41	30.25	26.51
1966	51.25	59.80	53.20	54.75
1967	55.37	43.66	55.50	51.51
1968	56.34	50.09	49.42	51.95
1969	47.53	48.87	70.70	55.70
1970	8.95	9.59	6.84	8.46
Average	36.12	36.46	36.21	36.26
Years high, %	25	38	38	—
Years medium, %	50	38	12	—
Years low, %	25	25	50	—

¹ Values are averages of analysis of all hogsheads sampled for indicated year: 8 hogsheads/grade/year in 1965 and 1967-70; 8 hogsheads each for grades X and C, 2 for grade B in 1963; 8 hogsheads for grade X and 6 each for grades B and C in 1964; 8 hogsheads each for grades X and C and 6 for grade B in 1966.

only 3.14 ppm in 1970 and below 0.3 ppm in 1971 and 1972. Residues on the 1972 crop from other areas also were much lower than in 1970, especially in areas I and II where total residues averaged over 20 ppm in 1971 but were 8 ppm and 6 ppm, respectively, in 1972.

It was particularly noteworthy that total DDT-TDE residues were so low in all 1972 tobacco (Table 5). These findings eliminated the possibility that the previous use of DDT and/or TDE for many years, and at high rates, would result in significant contamination of subsequent tobacco crops even after these insecticides were discontinued. Tables 4 and 5 show numerous instances in which DDT-TDE residues dropped to 1 ppm or less in tobacco produced in an area where, just the year before, the crop had contained over 20 ppm. This proved to be the case even in area II where the

TABLE 9.—Chlorinated insecticide residues, ppm, in tobacco sampled from auction markets in Kentucky according to position of leaves on plant¹

CROP YEAR	POSITION ON PLANT				PPM ON COM- POSITE SAMPLE ²
	TOP	MIDDLE	BOTTOM	AVERAGE	
1968	61.08	58.35	57.07	58.83	45.61
1969	48.61	63.10	59.09	56.93	53.61
1970	14.42	17.04	10.46	13.97	18.06
1971	9.92	6.68	9.21	8.66	9.00
1972	4.01	5.32	4.74	4.69	3.92
Average	22.61	30.10	28.11	28.60	26.04
Years high, %	40	60	0	—	—
Years medium, %	20	20	60	—	—
Years low, %	40	20	40	—	—

¹ Values are averages of analysis of all samples analyzed for indicated year.² Sample consisting of equal portions of top, middle, and bottom leaves.

residues were highest each of the past 10 years. In fact, the most dramatic reduction in any single year occurred in area II where total DDT-TDE residues were 80.7 ppm in 1969 and only 6.7 ppm in 1970.

Determining relative concentrations of the individual components comprising the total DDT-TDE residues showed that the ratios of the compounds varied considerably from sample to sample (Tables 4, 5). By averaging the results of all tobacco samples analyzed in this study, it was found that total DDT-TDE residues consisted of 50% *p,p'*-TDE, 30% *p,p'*-DDT, 12% *o,p*-TDE, 6% *o,p*-DDT, and 2% *p,p'*-DDE. Only after total residues on tobacco had declined to the point that minor components could no longer be detected, could a consistent variation from these average values be obtained. This had little meaning, however, since all five components were usually present in samples containing 0.5 ppm, and sometimes less, of either *p,p'*-DDT or *p,p'*-TDE.

Concomitant with the decline of DDT-TDE residues was the increased levels of endosulfan in tobacco. Maximum levels, up to 14 ppm, occurred in tobacco from areas I and II, but generally declined as the area of crop production moved eastward across the State. Area VI tobacco contained 0.55 ppm endosulfan in 1970, none in 1971, and only 0.12 ppm in 1972. Except for areas I and II, higher endosulfan residues were found in the 1972 tobacco than in the 1971 material.

Generally, the levels of dieldrin and endrin varied according to area of production and crop year in much the same manner as the DDT-TDE residues. By 1972, the levels had declined to a point where the highest level of dieldrin in any area was only 0.08 ppm (Table 5) and this occurred in area I. None of the 1972 samples from area III or VI contained dieldrin. Endrin residues of relatively high quantities, 0.43 ppm, were in the 1972 tobacco from area II, but were only 0.07 ppm in area I. All other samples were free of endrin residues in 1972. Of all samples collected from the auction warehouses, toxaphene was detected only in the 1969 area III and the 1970 area I tobacco.

ENDOSULFAN

It was evident from analyses of both the Burley Tobacco Pool and auction warehouse samples that many tobacco producers were using endosulfan as a replacement for DDT and TDE. Residues of endosulfan rose sharply in crops produced after 1969, the last year DDT and TDE were approved for control of tobacco insect pests. Because endosulfan is still approved for use on tobacco and was the predominant chlorinated insecticide residue on the 1971 and 1972 Burley tobacco crops, a more critical evaluation of its residual nature in tobacco is presented in Tables 6 and 7.

Total endosulfan residues consisted of the two isomers of the compound, endosulfan I and II, and endosulfan sulfate. Analysis of the three endosulfans on tobacco leaves from the top (B3F), middle (C4F), and bottom (X3F) of the plant showed that the bottom leaves contained the highest concentrations of total endosulfan residues. Generally, endosulfan residues were somewhat higher in the middle leaves than in the top ones. Although the differences were consistent, the magnitude was usually less than 1 ppm between the top and bottom leaves. Actually, it might be expected that the top leaves would contain the higher level of residues since endosulfan is applied as foliar spray to tobacco. It may be that residues on the lower portion of the plant are protected from weathering by the upper foliage, thus slowing dissipation rates. Another contributing factor could be dilution by growth of the upper leaves when younger plants are sprayed.

In most of the tobacco analyzed, endosulfan sulfate accounted for 40 to 50% of the total endosulfan residues.

Endosulfan II constituted 30 to 40% and the remaining 10 to 20% was endosulfan I. The 1972 tobacco crop, however, showed a different pattern (Table 7). Endosulfan sulfate was approximately 70% of the total residues; endosulfan II, about 20%; and endosulfan I, only 8%. Whether this is typical of newly harvested tobacco was not ascertained. However, it is unlikely that these data mean that significant changes in composition of endosulfan residues took place during storage of the 1969-70 tobacco samples. If this were the case, endosulfan sulfate residues should be less in newly harvested samples; they are formed from endosulfan I and II, and this conversion would have more time to occur in the stored tobacco. The fact that the relative concentrations of the endosulfan components were fairly similar on all tobacco suggests that little dissipation and/or conversion of endosulfan residues to other products occurred during storage.

RESIDUES IN RELATION TO GRADE OF TOBACCO

Tobacco stored in hogsheads by the Burley Tobacco Pool is graded before being placed in storage and this information is recorded on each container. Grades B3F, C4F, and X3F were sampled for residue analysis whenever possible because these grades generally represent the top, middle, and bottom leaves of the tobacco plant, respectively.

Samples from the auction warehouses were taken before being graded but the samples were taken based on the position of the leaves on the intact plant. Therefore, it was possible to correlate levels of residues with grades of tobacco in samples taken from the Burley Tobacco Pool and the auction markets.

The 8-year average chlorinated insecticide residue levels on the Burley Tobacco Pool samples were almost identical for all three grades of tobacco (Table 8). During this period, however, grades B and C contained the lowest level of residues only 25% of the time; grade X tobacco was lowest of the three grades 50% of the time. Residues on grades C and X were higher than grade B material 38% of the time. Grade B material was highest 25% of the time. During the years when residues were in the low and/or medium range, grade B tobacco was highest 75% of the time. These data do suggest a trend in regard to residue levels and grades of tobacco, but the differences are so small and inconsistent that it is impossible to consider them of any practical importance.

A similar conclusion may be drawn from data in Table 9 which show residue levels on the top, middle, and bottom leaves of tobacco sampled from auction warehouses. Average residue levels of chlorinated insecticides for crop years 1968 through 1972 were 23 ppm for the top leaves, 30 ppm for the middle leaves, and 28 ppm for the bottom leaves. As expected, these values were

less than the 8-year averages, 36 ppm, for tobacco stored in the Burley Tobacco Pool (Table 8). Calculating the frequency with which residue levels were either high, medium, or low on leaves from different positions of the tobacco plant failed to demonstrate a definite pattern of residue distribution (Table 9). The fact that these data on the auction market samples were different from but as variable as those for the Burley Tobacco Pool samples supports the conclusion that residue levels were independent of grade of tobacco.

Data in Tables 7 and 9 suggest that, for monitoring purposes, a composite sample consisting of equal quantities of top, middle, and bottom leaves would be a valid sample for analysis of the chlorinated insecticides. Actually, a sample taken from any one portion of the plant might serve the same function. However, if the apparent trend toward lower residues on top leaves is real as indicated by the data on endosulfan (Table 7), a composite sample would be of greater value in determining the level of residues on an entire tobacco crop.

It is significant that the findings of this study are limited to the chlorinated insecticides and that the distribution of other pesticides on tobacco could differ significantly. Soil-applied systemic pesticides, in particular, might be different from the chlorinated insecticides investigated in this study. With few exceptions, these latter materials

are applied as sprays directly on the plant and contamination is dependent largely on mechanical factors and ambient conditions. Contamination of tobacco by systemic insecticides would normally involve complex interactions of the chemical with various biochemical and physiological processes of the tobacco plant.

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BRIEF

Mercury Levels in Soils of the Eastern United States

G. B. Wiersma¹ and H. Tai²

ABSTRACT

Cropland and noncropland soils were sampled to determine levels of elemental mercury present in the upper three inches of soil. Results showed no difference in mercury levels between cropland and noncropland soils. Levels detected compared closely to levels found in similar studies. Actual mean levels of mercury residues in soils of the eastern United States ranged from 0.05 to 0.10 ppm.

Introduction

This study is an effort to determine baseline levels of elemental mercury in cropland and noncropland soils in the eastern United States. These baselines, in turn, will be used for comparison with results from continuing soil monitoring efforts for pesticides containing mercury.

Several estimates have been made of baseline levels of mercury in soils, both in the United States and abroad. Warren and Delavault (1) reported very high concentrations of mercury in certain British soils ranging as high as 15 ppm. They estimated, however, that the levels of mercury in most British agricultural soils would probably range between 0.01 and 0.06 ppm. Anderson (2) analyzed soils in Sweden and found that ranges for mercury in these soils varied from 0.02 to 0.92 ppm with an average of 0.07 ppm. Sand et al. (3) reported mercury residues in wheat-growing areas of the north-central United States.

Pierce et al., (4) reported mercury concentrations in soil from four areas in the western United States. The 50

percentile points for these four sets of data ranged from 0.071 ppm to 0.200 ppm. Shacklette et al. (5) collected 912 soil samples along widely scattered road systems in the continental United States. Sample sites were approximately 50 miles apart.

Sampling was conducted to the depth of 8 inches. The geometric means for mercury concentration in these soil samples for the entire United States was 0.071 ppm. The arithmetic mean was 0.112 ppm. The geometric mean for the western United States, 492 samples from west of the 97th meridian, was 0.055 ppm; the arithmetic mean was 0.083 ppm. The eastern United States, 420 samples, had a geometric mean of 0.096 ppm and an arithmetic mean of 0.147 ppm.

Material and Methods

Samples were collected as a subset of the National Soils Monitoring Program as described by Wiersma et al. (6). Sites were allocated according to the relative amounts of cropland and noncropland acreages in each State. Sites were chosen at random within the two categories. States sampled and the number of sites in each state are listed in Table 1.

At each sampling point a 10-acre site was sampled. Fifty soil cores, 2 inches in diameter by 3 inches deep, were collected and composited with the use of a 5-by-10 grid. A 2-quart subsample was then taken and sent to the laboratory for analysis. The cropland samples were collected in the fall of 1968, along with noncropland samples from Georgia, Maine, Maryland, Virginia, and West Virginia. All other noncropland samples were collected in the spring of 1971.

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TABLE 1.—*Soil samples collected to determine mercury residues in surface cropland and noncropland areas of eastern United States*

STATE	NUMBER OF SAMPLES COLLECTED	
	CROPLAND	NONCROPLAND
Alabama	6	6
Arkansas	13	5
Connecticut	1	1
Delaware	1	No samples tested
Florida	5	7
Georgia	8	8
Illinois	41	3
Indiana	22	1
Kentucky	8	4
Louisiana	8	5
Maine	2	2
Maryland	3	1
Massachusetts	1	1
Michigan	16	*
Mississippi	9	5
Missouri	23	7
New Hampshire	1	1
New Jersey	*	1
New York	11	5
North Carolina	8	6
Ohio	18	3
Oklahoma	19	7
Pennsylvania	11	5
South Carolina	5	3
Tennessee	8	5
Vermont	1	1
Virginia	7	4
West Virginia	3	2
Wisconsin	16	5
TOTAL	275	104

* No analyses available

Analytical Procedures

The analyses were done by a commercial laboratory in West Chester, Pennsylvania, under contract with the U.S. Environmental Protection Agency. The flameless atomic absorption spectrophotometry was applied for the determination of mercury.

Each soil sample, weighed exactly to 1.000 g, was dispersed in nitric acid solution (5 ml concd HNO_3 in 100 ml water). Digestion at 90°C , for approximately 2 hours, took place in the presence of excess potassium permanganate (portions of 1 ml 5% KMnO_4 solution were added until purple color persisted) and 2 ml of 5% potassium persulfate solution. After digestion, the mixture was cooled to room temperature and filtered. The filtrate was made to a volume of 100 ml, and 2 ml of sodium chloride-hydroxylamine reagent (12 g each in 100 ml water) was added to reduce the excess permanganate. Stannous sulfate solution (25 g in 250 ml of 0.5N H_2SO_4), 5 ml, was added to reduce mercury ions to elemental mercury, which was swept by an air stream through the absorption cell of the atomic absorption spectrophotometer. The absorptions were measured by peak height, and compared with a calibration curve.

A Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer with Automatic Null Recorder Readout was used for the measurements. The essential instrument conditions were:

Lamp: Mercury Hollow Cathode Lamp
(Westinghouse WL-22847)
Lamp current: 10 mA
Wave length: 253.7 nm
Slit setting: 3
Scale: $\times 1$
Noise suppression: 2

Precision study, using samples not reported in this paper, indicated a standard deviation of ± 0.16 at a level of $0.35 \mu\text{g/liter}$, with recovery average of 87%. The present procedure gave a detection limit of $0.05 \mu\text{g}$ mercury (or 0.05 ppm per 1-g sample).

Results and Discussion

After testing the distribution form of our data, we found that the data for mercury levels were not normally distributed with both positive skewness and significant kurtosis present. We found, however, that a logarithmic transformation helped the data to approach the normal distribution and to have a symmetric distribution. Shacklette et al. used logarithms to transform their data. They felt that the geometric mean was the best estimate of the central tendency of the data. We have found in our work similar results; therefore, we present both the arithmetic and geometric means. Table 2 shows the geometric means, 95% confidence levels, arithmetic means, the percent of samples that had detectable residues, and the detection range of mercury levels in both cropland and noncropland soils.

TABLE 2.—*Summary of mercury residues detected in surface cropland and noncropland soils of the eastern United States*

	ARITHMETIC MEAN, PPM	GEOMETRIC MEAN AND 95% CONFIDENCE INTERVAL, PPM	RANGE, PPM	PERCENT OF SAMPLES WITH DETECTABLE RESIDUES
Cropland	0.08	0.063 (0.069-0.058)	<0.05 to 1.06	100.0
Noncropland	0.07	0.054 (0.064-0.046)	<0.05 to 0.40	100.0

Mercury was present in all the samples collected at either a trace level or above. The difference between cropland and noncropland soils were not significant. This was determined from the overlapping confidence intervals. Although these samples were collected at different times, one would not expect mercury levels to change significantly from year to year.

Shacklette et al. have stated (5), "Surficial materials analyzed in this study are ordinarily sampled at a depth of 8 inches. We believe that soils and other regoliths from this depth commonly show little or no effects of surficial contamination that may have occurred." The samples in this study were collected to include possible

contamination of surface soil by pesticides and other pollutants containing mercury. If there had been a significant amount of mercury contamination from manmade sources that had reached soils through airborne or other routes, one would expect our samples to have considerably higher residues than those collected specifically to avoid surface contamination. However, the mercury levels reported in this study are actually lower than those reported by Shacklette et al.

To summarize: in mercury levels of the eastern United States there is no statistical difference between cropland and noncropland soils. The levels detected in this study agree closely with levels detected in similar studies. Actual mean levels of mercury residues in the soils of the eastern United States ranged from 0.05 to 0.10 ppm.

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APPENDIX

Chemical Names of Compounds Discussed in This Issue

BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
2,4-D	2,4-dichlorophenoxyacetic acid
DDE	1,1-dichloro-2,2-bis(<i>p</i> -chlorophenyl) ethylene
DDT (including its isomers and dehydrochlorination products)	1,1,1-trichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane; technical DDT consists of a mixture of the <i>p,p'</i> -isomer and the <i>o,p'</i> -isomer (in ratio of about 3 or 4 to 1)
DIELDRIN	Not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8a-octahydro-1,4- <i>endo-exo</i> -5,8-dimethano=naphthalene
ENDOSULFAN (THIODAN®)	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide
ENDRIN	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4- <i>endo-endo</i> -5,8-dimethanonaphthalene
HEPTACHLOR	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
HEPTACHLOR EPOXIDE	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4-7-methanoindan
MERCURY	Hg
MIREX	dodecachlorooctahydro-1,3,4-metheno-1 <i>H</i> -cyclobuta[<i>cd</i>] pentalene
POLYCHLORINATED BIPHENYLS (PCB's)	Mixtures of chlorinated biphenyl compounds having various percentages of chlorination
TDE (DDD) (including isomers and dehydrochlorination products)	1,1-dichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane; technical TDE contains some <i>o,p'</i> -isomer also
TOXAPHENE	chlorinated camphene containing 67-68% chlorine

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SUBJECT AND AUTHOR INDEXES

Volume 7, June 1973-March 1974

Preface

Primary headings in the subject index consist of pesticide compounds, the media in which residues are monitored, and several concept headings, as follows:

Pesticide Compounds (listed alphabetically by common name or trade name where there is no common name)

Media and Concept Headings

- Degradation
- Experimental Design
- Factors Influencing Residues
- Food and Feed
- Humans
- Plants (other than those used for food and feed)
- Sediment
- Soil
- Water
- Wildlife

Compound headings are also used as secondary headings under the primary media and concept headings and vice versa. When a particular paper discusses five or more organochlorines or three or more organophosphates or herbicides, the compounds are grouped by class under the media or concept headings; in the primary headings, however, all compounds are listed individually. The specific compounds or elements which have been grouped in various combinations by class for certain papers are as follows:

Organochlorines

- aldrin
- BHC/lindane
- chlordan
- DCBP
- DDE
- DDT
- dieldrin
- endosulfan
- endrin
- heptachlor/heptachlor epoxide
- mirex
- TDE
- toxaphene

Organophosphates

- diazinon
- malathion
- methyl parathion
- parathion

Herbicides

- 2,4-D
- silvex
- 2,4,5-T

In the author index, the names of both senior and junior authors appear alphabetically. Full citation is given, however, only under the senior author, with a reference to the senior author appearing under junior authors.

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